THE EFFECT OF NEUROSURGICAL ABLATION OF THE ENTORHINAL CORTEX

Ph. D. Thesis

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CONTENTS

ABBREVIATIONS

AChE	acetylcholinesterase
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANOVA	analysis of variance
AOI	area of interest
4-AP	4-aminopyridine
CA	cornu ammonis (Ammon's horn)
c-fos	cellular protooncoprotein
DAB	3'3'-diaminobenzidine tetrahydrochloride
DG	dentate gyrus
EC	entorhinal cortex
ECA	entorhinal cortex ablation
EEG	electroencephalography
Fos	oncogene encoded by the **F**inkel-Biskis-Jinkins murine **os**teogenic **s**arcoma virus
GABA	4-aminobutyric acid
GluR1-4	AMPA receptor subunits
GluR6,7	kainate receptor subunits
ip	intraperitoneal
KA	kainic acid
KA2	kainic acid receptor subunit
LEC	lateral entorhinal cortex
LECA	lateral entorhinal cortex ablation
MRI	magnetic resonance imaging
NMDA	N-methyl-D aspartate
NR1 and **NR2**	NMDA receptors subunits
PAP	peroxidase-antiperoxidase
PBS	phosphate-buffered saline
SDS	sodium dodecyl sulphate
SOC	sham operated control
TBS	TrisHCl-buffered saline
TLE	temporal lobe epilepsy

LIST OF JOURNAL ARTICLES

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. **Zs Kopniczky**, R Dochnal, M Mácsai, G Pál, Gy Szabó: Alterations of behaviour and spatial learning after unilateral entorhinal ablation of rats. *Life Sciences* (accepted for publication) **2005**

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II. **Zs Kopniczky**, E Dobó, S Borbély, I Világi, L Détári, B Krisztin-Péva, A Bagosi, E Molnár, A Mihály: Entorhinal cortex lesions rearrange afferents, glutamate receptors, increase seizure latency and suppress seizure-induced c-fos expression in the hippocampus of adult rat. *Journal of Neurochemistry* **2005**; 95(1):111-124.

IF:4.8

III. A Mihály, S Borbély, I Világi, L Détári, R Weiczner, Zs. Zádor, B Krisztin-Péva, A Bagosi, **Zs Kopniczky**, E Zádor: Neocortical c-fos mRNA transcription in repeated, brief, acute seizures: is c-fos a coincidence detector? *International Journal of Molecular Medicine* **2005**; 15:481-486.

IF:3.19

IV. **Kopniczky Zs**, Kóbor J, Maráz A, Vajtai I: Desmoplastic neuroepithelial tumor of infancy in the nevus sebaceus syndrome: report of a unique constellation and review of the literature. *Pathology Research and Practice* **2001**; 197(4):279-284.

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V. J. Miklossy, **Z. Kopniczky**, A Uske, F. Delacrétaz, P Chaubert., F Porchet: Lymphoplasmacyte-rich meningioma with follicular infiltrates. *Brain Pathology* **2000**; 10:477-483.

IF:6.435

VI. Vajtai I, Varga Zs, Bodosi M, **Kopniczky Zs**, Kóbor J, Vörös E: Dysembryoplastic neuroepithelial tumor. *Hungarian Medical Journal* **1995**; 48.2623-2627.

1. INTRODUCTION

1. 1. NEUROSURGICAL IMPLICATION OF EXPERIMENTAL STUDIES

A neurosurgeon may be interested to know more about epilepsy for two main reasons. First, the surgeon encounters and manages pathological conditions, which may induce seizures; these conditions are referred to as symptomatic epilepsies. Second, nearly one third of genuine epilepsies remain resistant to pharmacotherapy and neurosurgical intervention may be needed for better seizure control.

By definition, epilepsy is a chronic neurological disorder characterized by recurrent seizures of various forms and psychiatric concerns. The classification of seizures is fairly complex, however, two main groups are widely accepted: partial and generalized. Despite of the considerable advancement of anti-epileptic medical therapy, unfortunately, the overall drug-resistency of epilepsies is still 25-30%, so safe and efficient treatment is not always possible (14). A great number of epilepsies, most particularly the temporal lobe epilepsies (TLE) are accompanied by neuropathological alterations (IV, V, VI, 26, 53). In the clinical practice, the site of origin and the spread seizures must always be searched for. To decide about a pharmacological or a neurosurgical option of treatment, correspondences between the symptomatology, the EEG signs and the pathomorphological changes are examined regularly. If morphological data correspond well with clinical examinations, surgical intervention can be indicated with reasonable result of seizure control (14). The better understanding of the role of different brain regions in the development of seizures is essential for successful surgical interventions.

1. 2. MORPHOLGICAL ASPECTS OF EPILEPSY

Many brain regions and neural connections are involved in the epileptic mechanisms. More important are the neocortex proper, the thalamus and the thalamocortical connections, the temporobasal and parahippocampal regions, the numerous interhemispheric connections, especially the corpus callosum. Among these, the temporolimbic structures are particularly prone to elicit and maintain the epileptic seizures, that is why our studies focused primarily on these neuronal structures.

1.2.1. The temporal lobe and temporolimbic structures

The macroscopic anatomy of the temporal lobe is best conceptualized as a „truncated pyramid" with the temporal pole as the top and the base merging with the parietal and occipial lobes. It has four surfaces: lateral, ventral, medial (or mesial) and superior (or opercular) surface. The „hippocampal formation" is located in the ventromedial part of temporal lobe consisting of the parahippocampal gyrus, the hippocampus proper, the dentate gyrus and the subicular complex (58, 59).

From the very beginning of the postmortem anatomical studies of epileptic patients, pathological changes of several brain structures were documented. Initially, the term "sclerosis" was introduced to describe the macroscopical features of a hard, shrunken hippocampus by Bouchet and Cazauvielh in 1825. Sommer, in 1880, presented a case history of a patient suffering from temporal lobe epilepsy. He illustrated with microscopic histological slices the neural cell loss in the CA1 region of Ammon's horn and prosubiculum. Then, in 1899, Bratz precised and systematized the hippocampal lesions of various etiology and established the term ***„hippocampal sclerosis"*** consisting of severe neuron loss in Sommer's sector and in the endfolium (hilus and CA4), while the CA2 and CA3, like subiculum and other transitional cortices seemed to be „resistant" to damage. Other pathological changes in the ventromedial region of temporal lobes has also been well documented like atrophy, grey matter heterotopies or tumors associated to epilepsy. Physiological studies found that these region were especially prone for initiating seizures by different stimuli. In fact, the main stuctures supposed to play a role of epileptogenesis are: hippocampus, parahippocampal gyrus, amygdala, entorhinal cortex (EC), prefrontal area, fornix, cingulate gyrus. All of these structures constitute the so-called „temporo-limbic system", originally described as „le grand lobe limbic" by Broca in 1878. Several data suggest, that the EC has a central role in the normal function of the limbic system and in the epileptogenesis of TLE.

1.2.2. The entorhinal cortex (EC)

The human entorhinal cortex is the rostral (anterior) third of parahippocampal gyrus, defined by Brodmann (1909), as area 28. The name „entorhinal" refers to its location inside the rhinal sulcus, in the olfactory area, spatially associated with the amygdaloid complex rostrally and with the hippocampus caudally.

In fact, the transitional (mesocortical) character of this region is well reflected *histologically* by its cytoarchitectonic properties: unlike the *allocortex* (including the prepiriform and periamygdalar areas, the uncus and hippocampus), the *mesocortex* has six layers. The following layers have been identified by Insausti *et al.* in 1995 in primates (24):

I. Composed mainly of fibers and occasional cells;
II. Consisting of cell islands of polygonal and star-shaped neurons (like the islands of Calleja);
III. Composed of a superficial sublayer of small pyramidal neurons clustered together, and a deep portion of homogenously-arranged neurons;
IV. *Lamina dissecans*, a cell-sparse stratum between layer III and V;
V. Layer of darkly-stained pyramidal cells;
VI. Transitional zone towards the white matter.

Following the cytoarchitectonic differences and the connectivity to surrounding structures, several subfields of EC have been identified in the primate (5), which are difficult to compare to the subfields of the rat EC (76). In the rat two areas are discerned: the lateral entorhinal area (LEA) and medial entorhinal area (MEA). For our experimental work, the more accessible and more superficially located LEA was chosen to perform transcranial surgical ablation. This area was determined as the cortical surface right below the rhinal sulcus, running parallel to the temporal skull base, back to the transverse sinus, measuring 2 mm in length, 1 mm in width (dorsoventrally), and 1 mm thickness (6, 47).

1.2.3. Connections of the EC

- **Cortical and subcortical connections**

Two-thirds of the neocortical information projecting to EC is conveyed via the perirhinal, postrhinal and parahippocampal cortices, so these form the great majority of inputs to the EC (6). The peri- and postrhinal inputs to the LEA are stronger, overlapping and more concentrated, compared to those running to the MEA (65). Therefore, the influence of the LEA on the hippocampus is stronger, too. Most of the connections are reciprocal, thus prominent efferents from EC terminate in the referred cortical regions (65). The olfactory bulb is the only primary sensory (afferent) system directly projecting to EC, but there are dense afferent projections from infralimbic and orbitofrontal cortices, as well. Finally, insular and cingulate cortices are also innervating EC (65, 76).

From the *subcortical* level, the basal forebrain cholinergic fibers project to EC, serotoninergic fibers from the brain stem raphe nuclei, noradrenergic fibers from the locus coeruleus and ventral tegmental area also reach the EC (76). Additionally, thalamic afferents also innervate the EC from the nucleus reuniens and the anterior thalamic nuclei (6). These numerous connections project to various layers and subfields of the EC (6, 65, 76).

- **Hippocampal connections**

The most studied connections of EC are towards the hippocampus (61). The *perforant pathway* is considered to be the major route of communication between the EC and the hippocampus. This efferent connection is between layers II-III of the EC and the dentate gyrus (DG), plus the stratum lacunosum-moleculare of the CA1-3, stimulating the intrinsic circuitry of the hippocampal formation (59). The axons of layer II-III neurons from the EC impinge on the dendrites of the dentate granule cells in the outer portion of the molecular layer (59). The axons of the granule cells, called mossy fibers, innervate the dendrites of the pyramidal neurons of CA3, which in turn project to the CA1 region in the form of the Schaffer-collaterals (59). From CA1 and the subicular region the axons poject back to layer V of the EC, thus closing the entorhinal-hippocampal neuronal loop. It is worth knowing, that the axons of layer V EC neurons project back to those cortical-subcortical structures

which innervate the EC, thus closing this major neuronal chain, and connecting different areas of the neocortex, mesocortex and allocortex (6, 74). Most of the synaptic terminals within the hippocampus use glutamate, and strongly excite the hippocampal neurons, acting on postsynaptic AMPA and NMDA receptors (34). Thus, normally, the EC is an important relay station between isocortical and hippocampal areas. Within the anatomical organization of the medial temporal lobe structures the EC forms a bi-directional gateway, which receives and processes cortical polysensory informations, which are then forwarded to the hippocampus via the perforant path. The EC contributes to memory function, especially to the declarative, cognitive memory formation. Thus, the EC contributes to very complex learning processes, concerning mainly the spatial orientation of the subject or the animal. A recent functional MRI human study indicated, that the activity of EC represents the declarative memory encoding state, and predicts and determines whether learned experiences will be remembered or forgotten (13).

The structural alterations of the EC in epilepsy are well-known in the human, and also in experimental animals (IV, VI, 14, 26, 57). The important role of the human EC in the pathophysiology of temporal lobe epilepsy (TLE) is reflected by its characteristic shrinkage detectable with MRI on the side of the seizure focus in human (54). However, it is not clear whether if the neuronal loss of the EC was the cause or the consequence of the ongoing seizure process. There are also several questions as to the exact role of the EC in the precipitation, spread and maintenance of limbic seizure activity. In vitro experiments on combined EC-hippocampal slices suggested that the ictal activity was initiated in the EC and spread to the dentate area and the CA3 pyramidal cells synaptically (62). Slice experiments proved, that in vitro isolation of the EC did not prevent epileptic activity of the dentate granule cells, but attenuated the hippocampal seizure discharges (62). There are only a few in vivo experiments reporting similar electrophysiological effects of entorhinal ablation (63). Others stated, that the EC is a generator of ictal discharges, showing that the sectioning of the perforant path makes ictal discharges disappear in all regions of the hippocampus, but not in the EC (4).

1. 3. MODELING OF SEIZURES AND EPILEPSY

The very pioneering part of epilepsy research has been the experimental modeling of epileptic seizures. The two main types of models are: the acute and chronic models (11). While the chronic models are based on the aftermaths of experimental brain injury, the acute models give insight into the mechanisms which render the intact nervous system prone to convulsions. In acute models relatively brief periods of seizures are induced, with EEG phenomena resembling some aspects of epileptic seizures in human patients. By these means, partial and generalized types of seizures with

different behavioral patterns can be studied, depending on the type of the epileptogenic stimulus (focal or systemically applied, electrical, chemical, thermic etc...), and the animal chosen for experiments (Table 1). Interestingly, the first chemical „model" leading to epileptiform activity was the historical neurosurgical procedure of applying strychnine to a discrete area of cortical surface with a purpose of mapping neuronal functions. This substance produced a cortical "firing focus" within a few minutes, generating interictal-like spikes, thus serving as tracer of cortical connections. Similarly, the epileptic effect of penicillin, the first antibiotic powder applied on the cortical surface at the end of craniotomy was described by neurosurgeons.

The experimental procedures may facilitate the study of the following important problems and questions:

- The precise cellular (and molecular) mechanisms by which epileptiform activity is generated.
- The causes of the increased cell synchronization.
- The modulators that determine the time when a seizure erupts.
- The natural factors which suppress ictal activity and maintain the interictal state.
- The transition between interictal and ictal states.
- Does „primarily generalized" really mean a seizure eruption „everywhere", or there is a focal onset that simply cannot be identified?
- The preferred pathways of seizure spread. How do seizures stop?
- The role of active inhibitory neurons in the convulsion.
- The effects of antiepileptic drugs.
- The role of transmitters, their receptors in epilepsy.
- The expression/repression of genes following acute seizures.
- The long-term effects of repeated, brief seizures on nerve cells.

Table 1: Acute seizure models (from Engel, 11)

- Partial convulsions
  - Electrical stimulation
  - Topical/focal convulsants
- Generalized types of seizures
  - *Convulsive type:*
    - MES (maximal electroshock)
    - Maximal systemically administered convulsants
    - (e.g.: pentylentatrazole (PTZ), bicuculline, kainic acid, ouabain, ***4-aminopyridine***)
    - Fluorothyl seizures
  - *Absence type*
    - Subcortical electrical stimulation
    - Systemic low-dose PTZ
    - Feline generalized penicillin seizure
    - Intracerebroventricular opioids
    - Carbon dioxide withdrawal seizures
    - Hyperthermia in immature animals

1. 4. THE 4-AP MODEL OF EPILEPSY

For our experiments we have choosen the 4-aminopyridine (4-AP) induced acute seizure model. Aminopyridines are bioactive N-heterocyclic tertiary amines, which block the voltage-dependent K^+ channel. AP's are weak basis, thus they can exist in neutral and protonated forms at physiological pH. Experimental evidence suggests that the protonated form is bioactive. The neutral form crosses the lipid membrane, becomes protonated inside the cell, and then binds into the pore of the ion channel. The channel blockade precipitates well-described acute behavioural convulsions in different experimental animals (15, 37). The characteristic events develop regularly in 20-25 minutes, beginning with increased exploratory activity, followed by tremor of the vibrissal muscles, shivering and clonus of forelimbs. At the height of shivering, generalized tonic-clonic seizure (GS) develops, and lasts for 45-60 seconds. A quiet postictal period of 1-8 minutes follows, and the animals display GS again (39).

The elimination of the compound is following a standard kinetics and takes 2-3 h. From the brain tissue AP is getting into the CSF and excreted through the kidneys. The electrophysiology of 4-AP induced seizures has a large literature, because the coumpound is extensively used in *in vitro* slice preparations (62). Since 4-AP blocks the voltage-dependent potassium channels (the Ka–channel, or A-channel, which regulates the spike frequency in postsynaptic structures, and the Kv-channel, or delayed rectifier, which is involved in the repolarization phase of action potential), the prolonged action potential duration results in increased inflow of Ca^{++} into the presynaptic axons. Through the presynaptic voltage-sensitive Ca^{++} channels transmitter release is also facilitated, as reflected by

increased synaptic vesicle exocytosis at utrastructural level (69, 2). The latency of appearance of the first seizure is relatively short and constant (35). Seizures increase the regional cerebral blood flow (38), and cause hyperactivity and paroxysmal depolarization shifts in cerebrocortical neurons (1, 67, 49). The 4-AP is used for seizure induction both in vivo (37, 39, 40) and in vitro (38).

Thus, 4-AP acts on several hippocampal pathways releasing excitatory and inhibitory transmitters from synapses as proved by microdyalisis experiments (30, 48, 68, 73). Most of the excitatory synaptic terminals are glutamatergic, and glutamate acts on the various types of its receptors (27, 34). The two main groups are the ionotropic and the metabotropic receptors. Glutamate acting on ionotropic receptors will change the electrical property of the neuronal membrane, typically inducing action potential, while the effects of the metabotropic receptors are more complex: glutamate acting on metabotropic receptors regulates intracellular metabolic and signalling processes, and transmitter release. The rapid responses to glutamate at the synapses are mediated by postsynaptic AMPA-, kainate- and NMDA-type ionotropic receptors (iGluRs), summarised in Table 2.

Table 2: The classification of iontropic glutamate receptors

IONOTROPIC GLUTAMATE RECEPTORS			
GROUPS (named after agonists, see Abbreviations)	**SUBUNITS**	**CHARACTERISTICS**	
		Mechanism	**Localisation**
AMPA receptors	GluR1, 2, 3,4	Fast reacting, short lasting (Na^+, K^+, Ca^{++})	Every subfield of hippocampus
NMDA receptors (reflect the maturation of the brain)	NR1 NR2 A, NR2 B, NR2 C, NR2 D	Slow-reacting, long lasting (Na^+, K^+, Ca^{++})	Every subfield of hippocampus Embryonic type receptor
Kainate receptors (postsynaptic)	KA1, 2 GluR 6, 7	Co-acting with AMPA receptors, Na^+, K^+, Ca^{++}; action is slow, but mechanisms are largely unknown	Mainly the stratum lucidum of CA3 (mossy fibers)

1.5. THE AIMS OF OUR STUDIES

Based on the above-mentioned evidences about the central role of entorhinal cortex within normal circumstances and in the epileptogenesis, our present experiments were undertaken in order to study the consequences of unilateral EC lesion, namely:

1. To work out the unilateral LEA ablation of rats, suitable for being a "modified" model of experimental epilepsy.
2. To describe the short-term histological consequences of LEA ablation (3 days after surgery).
3. To characterize the effect of LEA ablation on 4-AP induced seizure onset and spreading by means of the neuronal c-fos expression in the hippocampus during acute in vivo experimental seizures.
4. To describe the electrophysiologial caractheristics of hippocampal regions after LEA ablation during the 4-AP induced epilepsy in freely moving animals, by means of deep brain electrodes.
5. To determine alterions of epileptogenicity, spontan behaviour and spatial learning processes of rats after LEA ablation.
6. To describe phenomena of neuroplasticity in terms of sprouting and changes of expression of different types of ionotropic glutamate receptors in the chronic phase of LEA ablation.

2. MATERIALS AND METHODS

Adult, male Wistar rats (150-180 g bwt) were used for the experiments (Table 3), which were conducted in accordance with prevailing laws and ethical considerations, according to the directive of the Eueopean Council (86/609 EEC) and to the Hungarian Animal Act.. Written permission was obtained in advance from the Faculty Ethical Committee on Animal Experiments (University of Szeged).

2.1. Neurosurgical technique of unilateral entorhinal cortex ablation (LECA)

The animals were anesthetized with Calypsol® (100 mg/kg) plus atropine (0.01 mg/kg) given intraperitoneally (ip). The head of the animal was fixed in a stereotaxic frame, and following a vertical skin incision, the soft tissues and the temporal muscle were cut and kept aside in order to expose the temporo-basal region of the skull on the left side. The bone was cut and removed with a dental drill, a temporo-basal craniectomy was performed just before the transverse sinus and rhinal sulcus was identified. The lateral area of the entorhinal cortex (LEC) can be found inferior to the rhinal sulcus down to the temporal basis and back to the transverse sinus (6). The LEC was electrocoagulated and aspirated with micropipette-suction. Once, the ablation was achieved, a little

piece of temporal muscle was laid on the surface of cortical excision for ensuring safe hemostasis. Afterwards, the bone defect was covered with layers of the temporalis muscle and fascia, finally the skin was closed with clamps. In the sham-operated animals of controls (SOC) the same procedure was performed except for coagulation and aspiration of the cortex, the meninges and the brain were not manipulated.

Table 3. Overall data of animals treated in the experiments

			NUMBER OF ANIMALS		
Groups	**Studies performed**		**Operated**	**Sham operated**	**Total number**
A	Early histological consequences of LECA		3	3	**6**
B	Behaviour and spatial learning studies after LECA		33	12	**45**
C	EEG studies of 4-AP induced epilepsy after LECA		7	6	**13**
D	c-fos immunohistochemistry studies of 4-AP induced epilepsy after LECA		6	3	**9**
E	Late histological consequences of LECA	AChE histochemistry	3	3	**6**
		Calretinin immunohistochemistry	3	3	**6**
		In situ histoblotting for glutamate receptors	4	4	**8**
					93

2.2. Methods used in different animal groups

GROUP 'A'

The 3 LECA and 3 SOC rats were investigated with **immunohistochemical techniques 3 days after the surgery**. The animals were anesthetized with diethyl ether and perfused through the heart with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were kept in fixative overnight, then washed in phosphate buffered saline (PBS, pH 7.4), cryoprotected in 25% sucrose, and

sectioned on a freezing microtome at 25 μm. The tissue sections were processed for the immunohistochemical detection of synapsin I (rabbit anti-synapsin I, Alexis Corporation; 1:1000 dilution) and microglia antigen (mouse anti-rat CD11b, clone MRC OX-42, SEROTEC; 1:100 dilution), using the streptavidin-peroxidase method with 3'3'-diaminobenzidine tetrahydrochloride (DAB) substrate.

GROUP 'B'

Open field test

The number of animals tested in open field was 44 (32 LECA, 12 SOC). The open-field apparatus is a square shaped open field cage with a side length of 60 cm, surrounded by a 0.4 m high wall. The floor of the cage is divided in 36 (6x6) small squares. A 60 W light is situated 1 m above the arena floor. The procedure sessions started at 9 a.m. All animals were carried to the experimental room in their home cages. They were handled without gloves. Each animal was placed in the center of the open-field and was observed for 5 minutes.

The locomotor activity (horizontal activity) as number of squares crossed and rearing frequency (vertical activity) as number of times the animals stood on their hind legs was evaluated. Furthermore, defecation and grooming was also observed.

Elevated plus-maze

The number of rats examined was 21 (16 LECA, 5 SOC). The apparatus used in the elevated plus maze consists of two closed arms (10x40 cm) and two open arms (10x40 cm) forming a cross, with a quadrangular center (10x10 cm). The maze is placed 50 cm above the floor. A 60 W light is situated 1 m above the plus-maze apparatus. Sessions started at 9 a.m. All animals were carried to the experimental room in their home cages. They were handled without gloves. The rats were placed on the center facing an open arm. Each animal was observed for 5 minutes. The combination of height, luminosity and open space is assumed to induce fear or anxiety in the animal. The degree of anxiety is assessed by measuring the time spent on the open vs. closed arms and the number of entries into each arm.

Morris water maze test

The number of animals tested in water maze was 29. The setup consists of a round water pool (2 m in diameter) containing opacified water (temperature: 26°C) with a hidden escape platform. The subject must learn the location of the platform using contextual, local and distant cues. Theoretically the

water pool is divided into four quadrants, small platform, big platform, and annulus (Fig. 1. A and B).

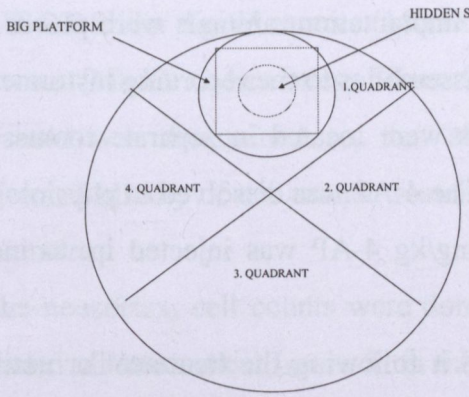


Fig. 1 A: The setup of water maze

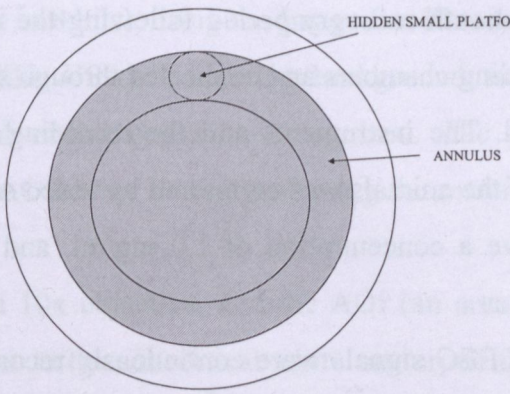


Fig. 1 B: The setup of water maze

During the training period, animals were tested 2 times a day (morning session - from 9.00 to 11.00 and afternoon session from 13.00 to 15.00) for five consecutive days. During one session a rat was placed in one quadrant of the pool and allowed to swim until the small platform was found. Rats were left on the platform for 15 seconds. If the platform was not found within 2 minutes, the animals were directly placed on the platform for 15 seconds. The starting point was randomly rotated for every trial. The hidden platform was located in a fix position with respect to the surrounding area.

After the training period, a test was administered on day 6 (reference day). During this trial the platform was removed from the pool and the swimming path was recorded for 2 minutes for each animal. The animal behavior was tracked by a video camera and analyzed by video software. The events registered were as follows: swimming speed, distance traveled by, latency to reach the platform, entrances and time spent in the goal area (small platform and the surrounding area), entrances, and the time spent in the annulus.

Statistical analysis of the data was made by repeated measure analysis of variance (ANOVA) and paired *t*-test. For significant ANOVA values, groups were compared by Tukey's test for multiple comparisons with unequal cell size. A probability value less than 0.05 was considered statistically significant.

GROUP 'C'

The 6 LECA and 3 SOC animals were treated for **electrophysiological studies**. After the surgery, the animals recovered, and survived for 40 days. Then, they were deeply anaesthetised with Nembutal (50 mg/kg, ip.) and fixed in a stereotaxic head-frame. Stainless steel screw-electrodes were implanted over the EC, and deep bipolar electrodes into the hippocampus (P 3.3, L 2.0, V 3.0) on

both sides, according to the stereotaxic atlas of Paxinos and Watson (47). An additional screw electrode over the cerebellum served as a reference point. The electrode leads were attached to a miniature socket fixed to the skull with dental acrylic cement.

After a one-month recovery period following the electrode implantation, animals were placed into plastic recording chambers and connected through slip-ring assemblies to the recording instrument to be registered. The instruments and the recording chambers were located in separate rooms. The behaviour of the animals was monitored by video cameras. The 4-AP was dissolved in physiological saline to have a concentration of 1.0 mg/ml, and then 5 mg/kg 4-AP was injected ip. to induce seizure.

Spontaneous EEG signals were continuously recorded for 8 h following the treatment or until the animal died in seizures. Signals were amplified 1000-fold with a commercial multichannel amplifier, filtered between of 0.3-100 Hz, digitised at a sampling rate of 100 Hz, and stored on a personal computer for off-line analyses. Epileptiform discharges were analysed by a custom written software. The latency, the duration, and the frequency of the seizures were determined visually on the basis of behavioural changes and of the analysis of EEG recordings. At the end of experiments, surviving animals were sacrificed with an overdose of Nembutal to investigate the position of the electrodes.

After testing the assumptions a 3-way ANOVA variance analysis was performed, followed by a Newman-Keuls post hoc comparison test.

GROUP 'D'

The 6 LECA and 3 SOC animals received intraperitoneal injection of 4-AP (5 mg/kg) without anesthesia, and were closely observed for epileptic behavioural or tonic-clonic activities. 3 h after the induced convulsions the animals were perfused through the heart with buffered 4% paraformaldehyde solution, and the brains were processed for **c-fos immunohistochemistry**. Polyclonal c-fos antibody (raised in rabbit; Santa Cruz Biotechnology, CA, USA) and the peroxidase-antiperoxidase (PAP) method were used. The sections were pretreated with 1.5% H_2O_2 and and rinsed in 0.1 M PBS containing 0.2% Triton X-100. They were incubated in 20% normal pig serum, next in primary c-fos antibody (1:1000 in 20% normal pig serum in PBS and 0.2% sodium azide), and then in donkey anti-rabbit IgG (1:40; Jackson Immuno-Research, PA, USA). The secondary antibody was detected by the PAP technique (PAP complex diluted to 1:1000). The peroxidase reaction was localised with nickel chloride-containing DAB (Sigma), yielding a black reaction product.

Quantitative analysis was performed on 5 sections per animal, selected from every brain on the basis of the same stereotaxic coordinates. Areas of interest (AOIs) for counts of immunostained neuronal

nuclei were selected from the S1Tr region of the neocortex, regions CA1, CA2 and CA3 of the Ammon's horn, and from the hilus and granule cell layer of the dentate gyrus (47). Within each AOI, the immunoreactive cell nuclei were counted using a Nikon Eclipse 600 microscope equipped with a SPOT RT Slider digital camera (1600 x 1200 dpi in 8 bits), using the Image Pro Plus 4 morphometry software (Media Cybernetics, Silver Spring, MD, USA). Following background subtraction, the threshold was determined so, that all labelled nuclei could be recognized. The counting was performed blindly of the animal treatment. The AOIs were determined using the rectangular field of the camera.

In the neocortex, cell counts were done using a 10x objective, and the AOI (an area of 1.2 mm^2) included all neocortical layers from the pia mater to the subcortical white matter, so that the layers were not evaluated separately. Cell counts were then normalised to 1 mm^2. In another series of measurements, the same histological sections were used, and the neocortical layers were analysed separately. Frozen sections of the same thickness, stained with cresyl violet were used as a reference for the neocortical layers (76). The laminar cell counts were obtained with a 40x objective magnification. In the hippocampus, cell counts were done using a 40x objective, and were again normalized to 1 mm^2. In regions CA1-3, the AOI (an area of 0.05 mm^2) included the stratum pyramidale and a narrow zone of the strata oriens and radiatum. The hilus of the dentate gyrus was outlined, and counting was performed. In each section we measured the section thickness with 40x DIC dry objective using Marzhauser MultiControl 2000 motorized stage mounted to microscope and Scope-Pro Plug-In Module for Image-Pro Plus 4.5.

The real thickness was rectified for a refractive index due to dry objective. Finally cell counts were normalised to 1 mm^3 of tissue, and analyzed by means of independent samples *t*-test, the mean values of the cell numbers of the operated and contralateral sides were compared at a 0.05 significance level. Statistical analysis was done with the SPSS 9.0 computer program.

GROUP 'E'

AChE histochemistry

The animals (6 ECA and 6 SOC rats) were sacrificed 40 days after the surgery, and 25 μm thin frozen sections were processed for acetylcholinesterase (AChE) histochemistry (3-3 animals) and calretinin immunohistochemistry (3-3 animals). Following perfusion fixation, the brains were removed, and immersed in the fixative overnight at 4°C, then infiltrated with 30% sucrose in 100 mM Tris-HCl; pH 7.8. Serial horizontal-plane sections were cut at 25 μm on a freezing microtome, collected in TBS, and kept in a refrigerator until processed. Free-floating sections were pretreated in a solution containing 3% H_2O_2 and 3% Triton X-100 in TBS for 10 min. Then, the sections were

rinsed in TBS three times, and treated in 0.2 mM ethopropazine (Sigma) in the dark for 30 min to inhibit the non-specific cholinesterase activity. Following three rinses in PB, the sections were incubated in a solution, made with PB, of 1/15th dilution of the medium of Karnovsky and Roots (29) for 30 min at room temperature. After washing in three changes of TB, the reaction end-product was detected in a solution containing 0.05% DAB and 0.005 % H_2O_2 in TB. The sections were mounted on glass slides, and covered with Entellan®.

Calretinin immunohistochemistry

Calretinin immunohistochemistry was performed on horizontal-plane, free-floating frozen sections, using the streptavidin-peroxidase method, DAB substrate and nickel-chloride intensification. Primary antibody (rabbit anti-calretinin; Zymed Laboratories Inc., California, USA) was used in 1:5000 dilution. The optical density of AChE -positive nerve fibers was measured on digitalized images with help of the Image Pro Plus software. The number of calretinin-positive nerve cells in the dentate gyrus was counted with the same software (see the method of cell counting int he previous paragraph). The optical density of the calretinin-immunoreactive nerve fibers was measured in the molecular layer of the dentate gyrus. Statistical analysis was done by the SPSS 9.0 computer program.

In situ histoblotting technique

Animals of this subgroup comprised 4 ECA and 4 SOC rats, which were sacrificed 40 days after the surgery. They were deeply anesthetized with diethyl ether, decapitated, the brains were quickly frozen in isopentane, and were then stored at -80°C until sectioning. Cryostat sections (10 μm thin) were cut in the horizontal plane, and mounted on glass slides. The sections were stored at -20°C. The glutamate receptor subunits were localized by means of the in situ histoblotting technique (70). In brief, the cryostat sections were apposed to nitrocellulose membranes, which were previously moistened with 48 mM Tris-base, 39 mM glycine, 2% (w/v) SDS and 20% (w/v) methanol for 15 min at room temperature. After blocking in 5% (w/v) non-fat dry milk in PBS, nitrocellulose membranes were DNase I-treated (5 U/ml), washed and incubated in 2% (w/v) SDS, 100 mM β-mercaptoethanol in 100 mM Tris-HCl (pH 7.0), for 60 min at 45°C to remove adhering tissue residues. After excessive washing, the blots were reacted with affinity-purified anti-GluR1-4, anti-NR-1 and anti-KA-2 antibodies (0.5 μg/ml) in blocking solution overnight, at 4°C. The bound primary antibodies were detected with alkaline phosphatase-conjugated anti-rabbit IgG secondary antibody (70). To facilitate the identification of structures and cell layers, some cryostat sections were stained with cresyl violet. Digital images were acquired by scanning the membranes using a

desktop scanner. Image analysis and processing were performed on Adobe® Photoshop® software. When processing the images, each was treated identically to allow comparison. Initial data were collected as pixel density values in the hippocampal regions shown in Fig. 6. (= Fig. 11) Data were analysed and plotted using a GraphPad® Version 4.0. Differences between ECA and SOC animals were compared using a two-way analysis of variance (ANOVA), and further compared with the Bonferroni post hoc test, at a minimum confidence level of $p<0.05$.

3. RESULTS

3.1. Histoanatomical description of early consequences of the LECA

The hematoxylin-eosin stained sections allowed to confirm that surgery performed previously really resulted in the destruction of the lateral entorhinal cortical area (Fig. 2 A,B). Further histological analysis of the effect of the EC lesion showed the dropout of synapsin I immunoreactivity in the molecular layer of the dentate gyrus and in the stratum lacunosum-moleculare of regions CA1-3 on the side of the operation (Fig. 2 ~~E,F~~ C,D). In the same location strong microglia proliferation was observed (Fig. 2 ~~C,D~~ E,F). No significant neuronal loss, or other neuropathological phenomenon were detected in the hippocampus of the operated side (Fig. 2A,B; 8C).

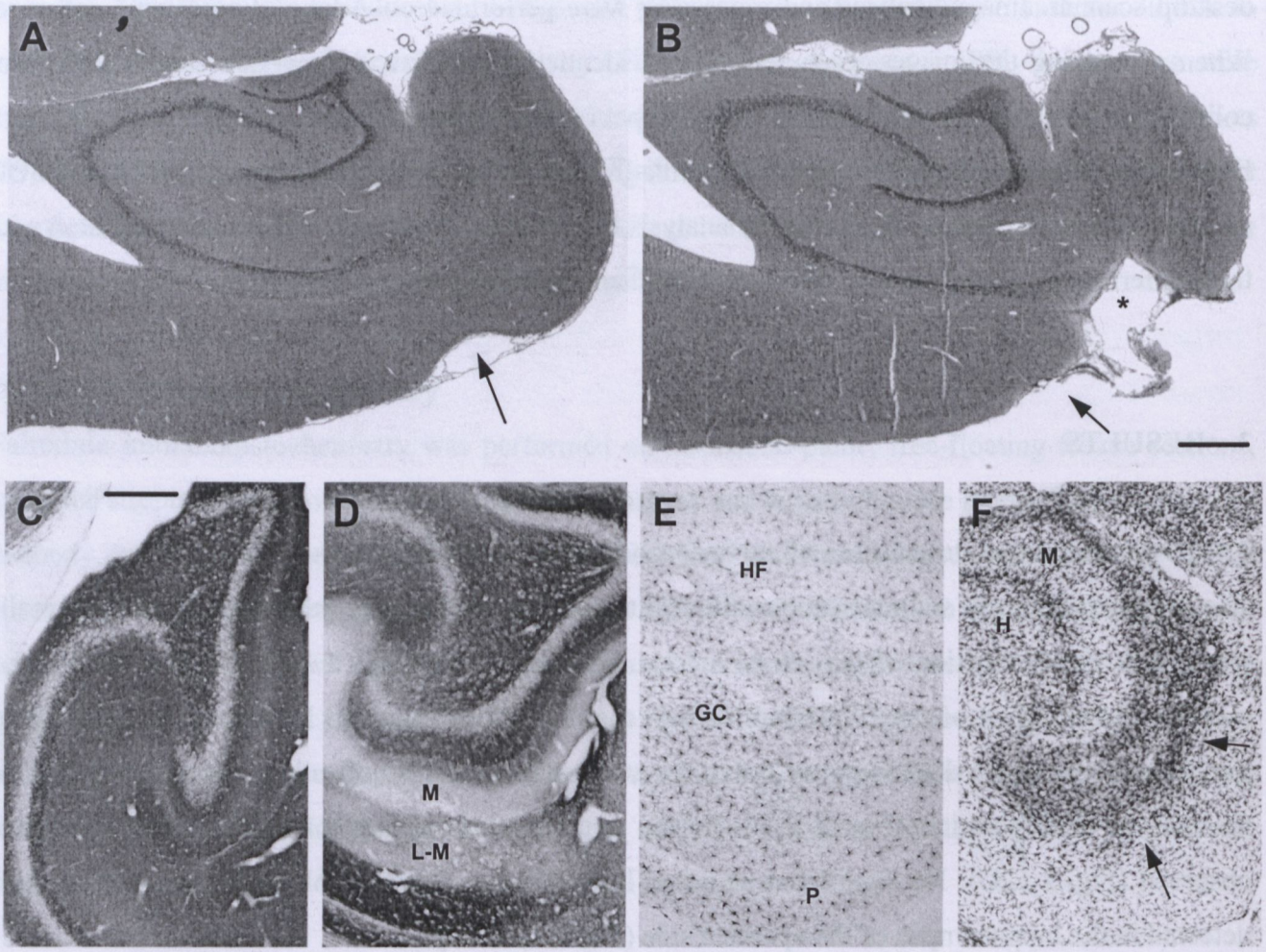


Fig. 2: Early consequences of left-sided EC ablation of adult male Wistar rats. (A,C,E,G: non-operated; B,D,F,H: after EC-lesion)

A and **B**: hematoxylin-eosin stained paraffin embedded thin sections: arrow points to rhinal sulcus, asterix shows the lesion; bar:1mm.

C and **D**: mouse anti-rat CD11b stained microglia in the hippocampus, on the side of the lesion arrows sign the strong microglia proliferation (arrows) of the ending area of perforant path.

E and **F**: terminal degeneration of perforant path proved by dropout of synapsin I immunoreactivity in the molecular layer (M) of the dentate gyrus and in stratum lacunosum-moleculare of regions CA1-1 (L-M) on the side of the operation. GC: granule cell layer; HF: hippocampal fissure; P: pyramidal cell layer; H: hilum. Bar: 250 μm.

3.2.Behaviour and spatial learning following LECA

First, we can declare, that during the time of observation no spontaneous epileptic behaviour was detected on rats with EC ablation, and no significant changes between EEG recordings of operated and control groups were found. The data acquired about spontaneous exploratory activity in open field test showed, that ablation of the entorhinal cortex results in a significant increase of both horizontal and vertical locomotor activity (Fig. 3) compared to the control group. However, the rate of defecation and grooming did not change.

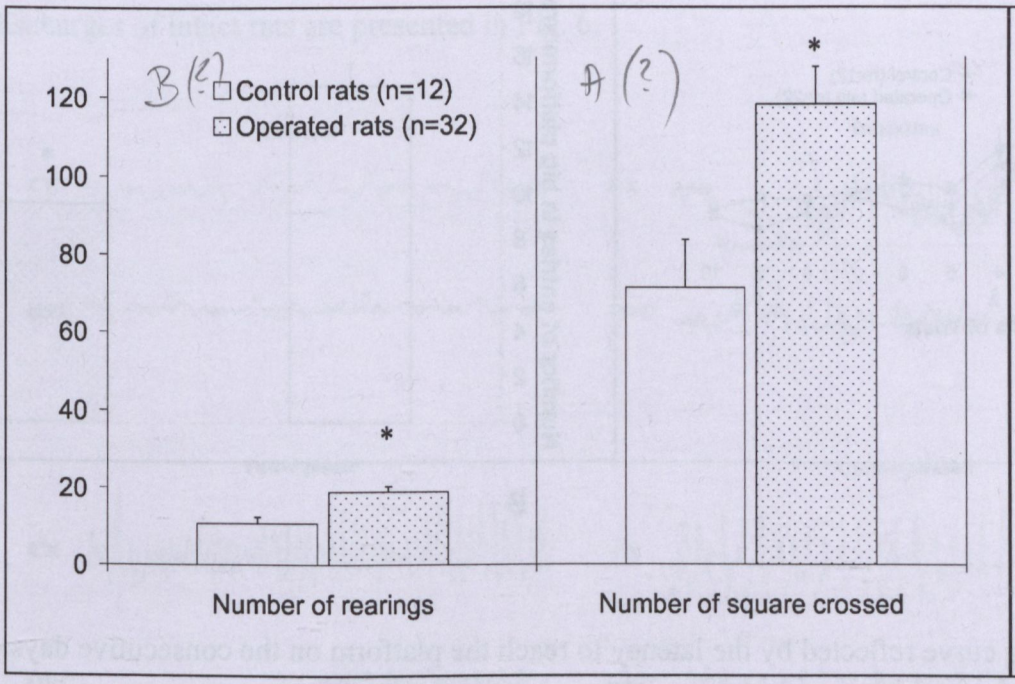


Fig. 3.: Horizontal locomotor activity (A) and vertical locomotor activity (B) were assessed with open field test. Data are given as mean ± S.E.M.; * P <0.05 vs. control.

In the elevated plus-maze test operated rats showed more open arm activity than the control animals (more entries in the open arms and more time spent in the open arms). These results were not significant (Fig. 4).

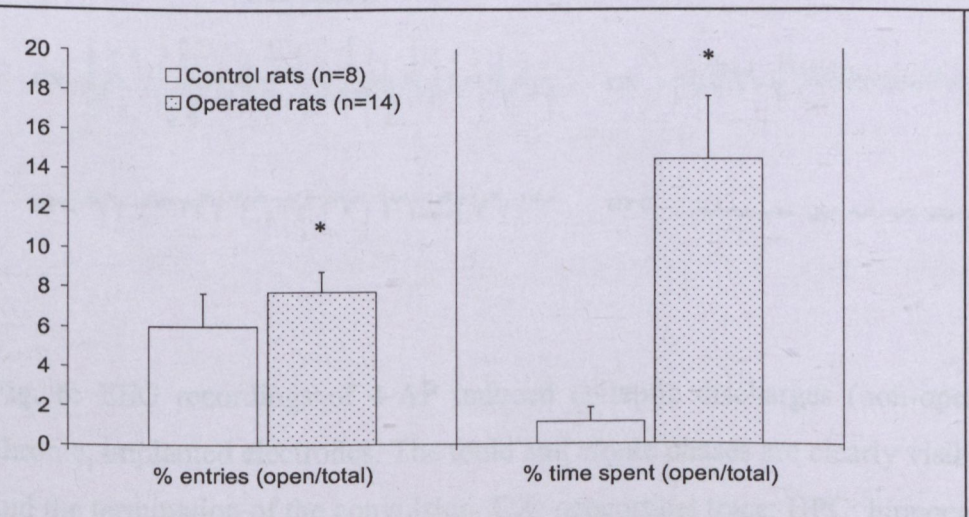
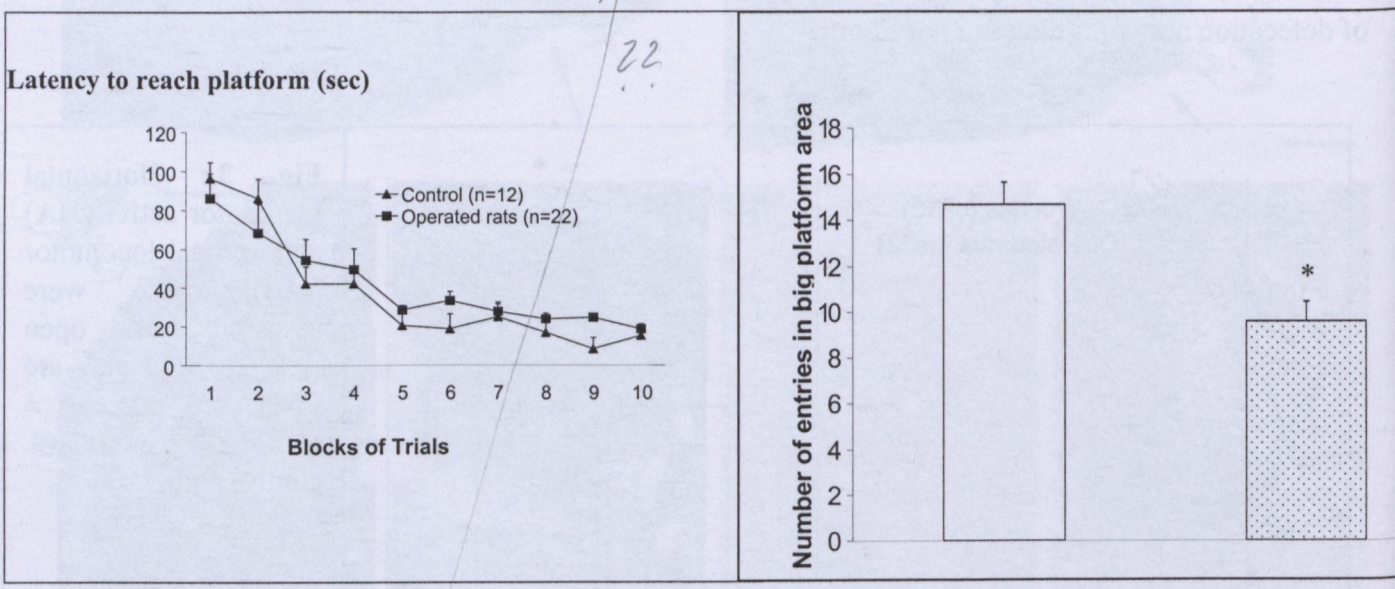


Fig. 4. Operated animals showed more open arm entries and spent more time in the open arms in the elevated plus maze test. Vertical lines on the top of the bars denote the S.E.M. * P<0.05 vs. control group.

Results of Morris water maze did not prove difference in terms of speed of swimming, or of distance traveled by, between the two tested groups. The rate of entrances and the time spent in the goal area (small platform and the surrounding area) was clearly less in the operated group of rats. The same way, operated group entered fewer, and spent less time in the annulus region than the control group. However, all of these changes are not significant statistically (Fig. 5A and B).



A B

Fig. 5 A: The learning curve reflected by the latency to reach the platform on the consecutive days of trials. No significant differences were detected. **B:** Number of entries in big platform area on the day of trial was significantly less in entorhinal cortex operated rats. Data are given as mean ± S.E.M. *P<0.05 vs. control group.

3.3. Electrophysiological studies in LECA during 4-AP seizures

Following ip. administration of 4-AP, generalised tonic-clonic seizures occured in every animal. In each animal of the control group (n=6) two seizures were detected during the 8 h long recording period. In the operated group there was only one seizure or no seizure at all (3 out of 7 animals). More than two seizures appeared in 1 animal of each group, the last seizure was lethal for them. At the beginning of the seizure events rats were running quickly along the wall of the chamber, and the characteristic tonic phase of the EEG activity could be detected. During the clonic phase of the EEG activity animals were laying on their side. EEG recordings of the typical 4-AP induced epileptic discharges of intact rats are presented in Fig. 6.

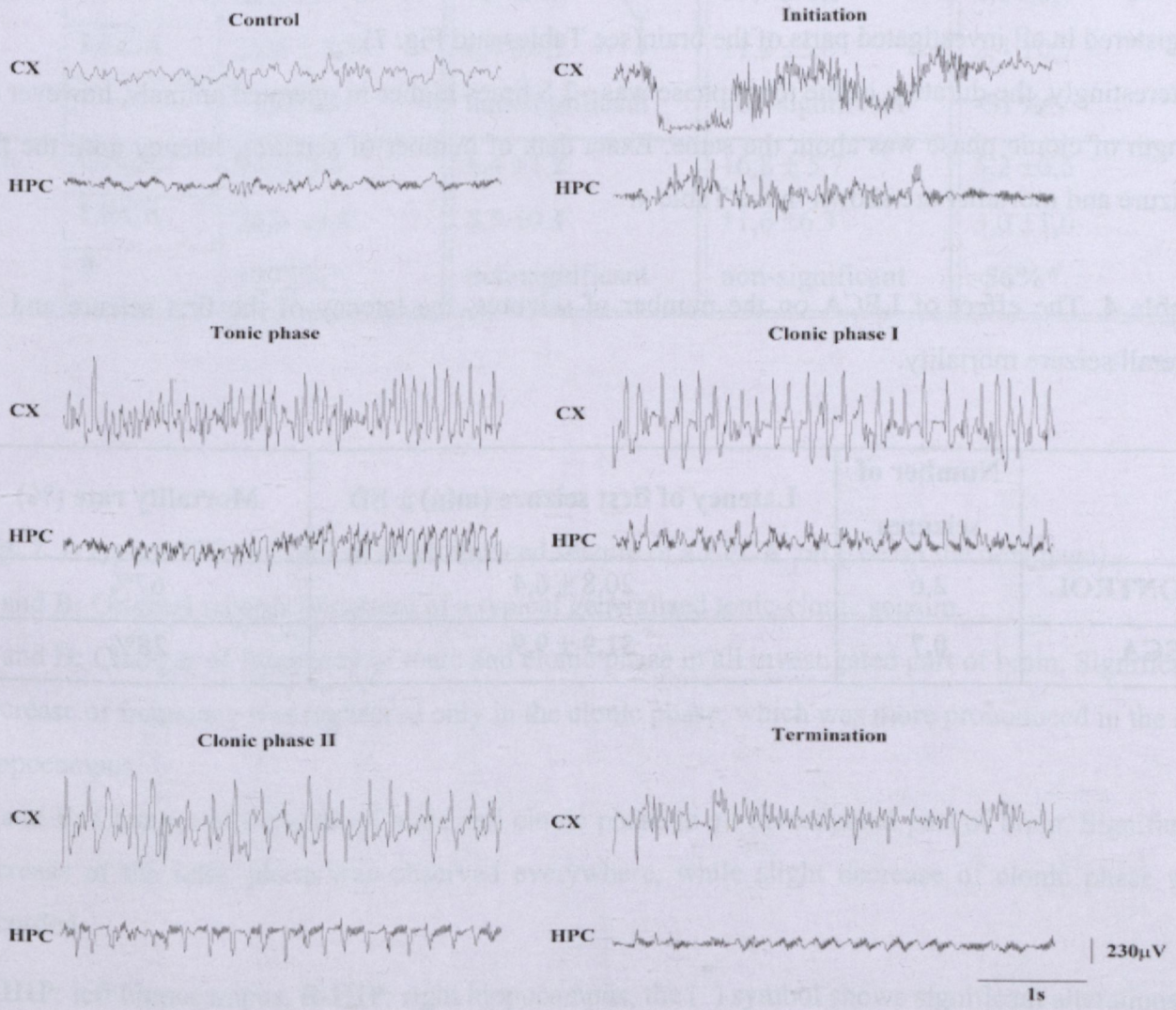


Fig. 6: EEG recordings of 4-AP induced epileptic discharges (non-operated rats), obtained with chronic, implanted electrodes. The tonic and clonic phases are clearly visible, as well as the initiation and the termination of the convulsion. CX: neocortical trace; HPC: hippocampal trace.

The mortality rate of LECA animals showed a 40 % decrease. The first seizure appeared during the first 30 min in the non-operated group, with an average latency of 20.82±6.43 min. These events were followed by a second seizure within the first hour after the treatment with the convulsant. The mean delay between the two seizure activities were 16.22±4.86 min, while the second was significantly longer, lasting for 73.44±13.94 seconds. In the LECA group the latency of first seizure increased up to 31.86 min and only one rat presented a second epileptic event (Tables 4 and 5).

Each seizure was characterised by repetitive spike–frequencies in the tonic and clonic phase, "spike and wave" complexes were also found, respectively. Differences of EEG properties were compared between control and operated animals. In terms of frequency, there was no significant difference during the tonic phase, whilst significant decrease (~50 %) during clonic phase could have been registered in all investigated parts of the brain (see Tables and Fig. 7).

Interestingly, the duration of the tonic phase was ~2.5 times higher in operated animals, however the length of clonic phase was about the same. Exact data of number of seizures, latency until the first seizure and mortality are shown in the Table 4.

Table 4. The effect of LECA on the number of seizures, the latency of the first seizure and the overall seizure mortality.

	Number of seizures	**Latency of first seizure (min) ± SD**	**Mortality rate (%)**
CONTROL	2,6	20,8 ± 6,4	67%
LECA	**0,7**	**31,9 ± 9,9**	**28%**

Table 5.: Data measured during 4-AP induced epileptic seizure (lHc: left hippocampus; rHc: right hippocampus; lErh: left entorhinal cortex; *: Significant change; P ≤0.001)

Electrode position		TONIC PHASE		CLONIC PHASE	
		Duration (sec)	**Frequency (Hz)**	**Duration (sec)**	**Frequency (Hz)**
lHC	Control	9,3 ± 2,4	6,6 ±1,2	17,7 ±4,4	4,9 ±0,2
	LECA	22,4 ±7,4	6,00 ±0,8	14,5 ±2,5	2,1 ±0,6
	Δ	**+240%***	non-significant	non-significant	**-43%***
rHC	Control	11 ±4,2	6,6 ± 0,9	16,7 ± 3,5	4,6 ±0,4
	LECA	20,6 ± 8,5	6,0 ±0,7	11,2 ±5,6	2,4 ±0,7
	Δ	**+187%***	non-significant	non-significant	**-51%***
lErh	Control	9,0 ± 3,4	6,4 ±1,2	16,8 ± 5,7	5,2 ±0,5
	LECA	24,9 ±9,8	5,5 ±0,4	11,6 ±6,3	3,0 ±1,6
	Δ	**+277%***	non-significant	non-significant	**-56%***

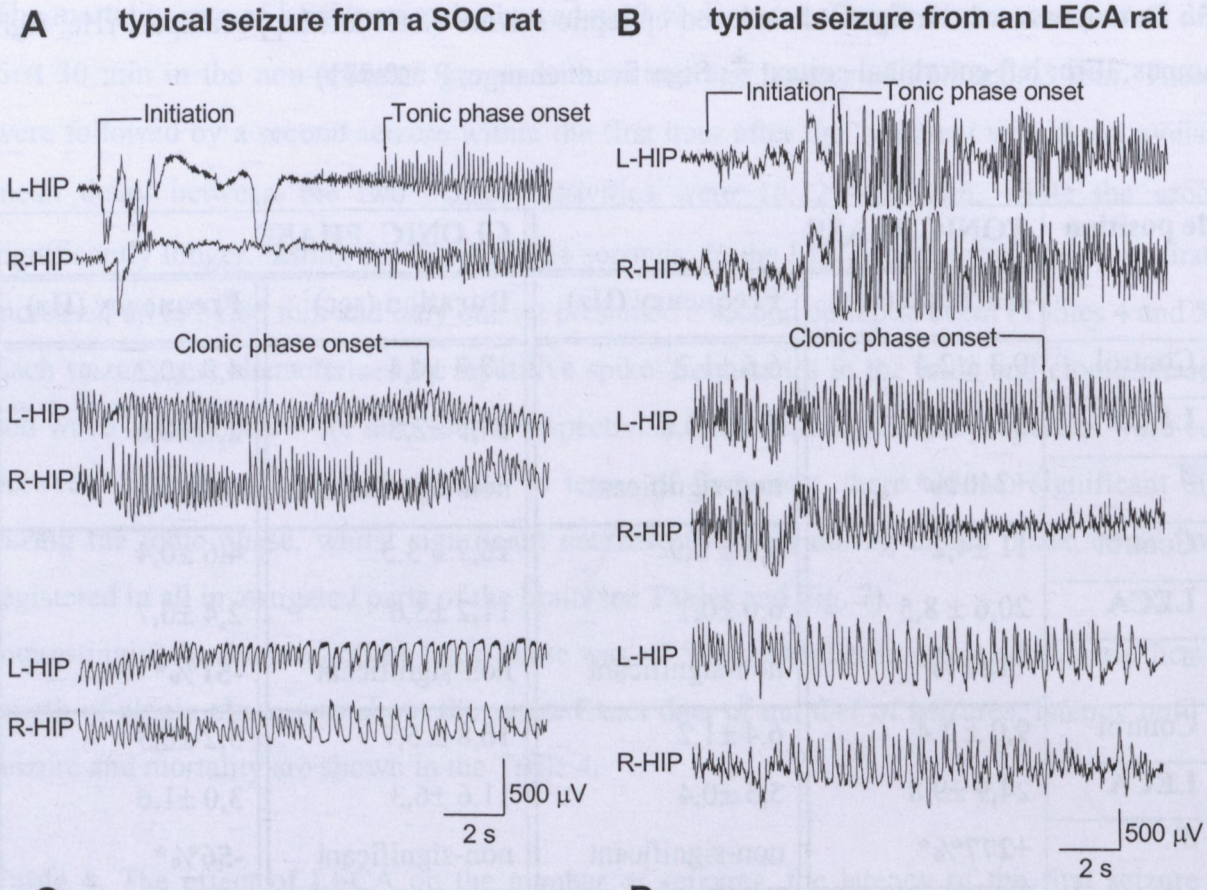
Fig. 7. A typical EEG records of 4-AP induced seizure of a LECA rat (see on the next page).

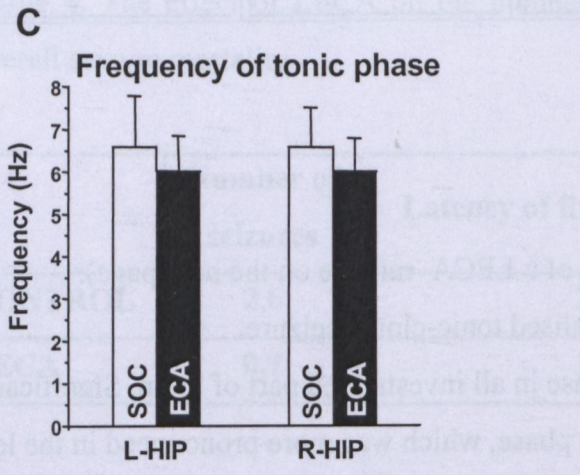
A and **B:** Original records of pattern of a typical generalised tonic-clonic seizure.

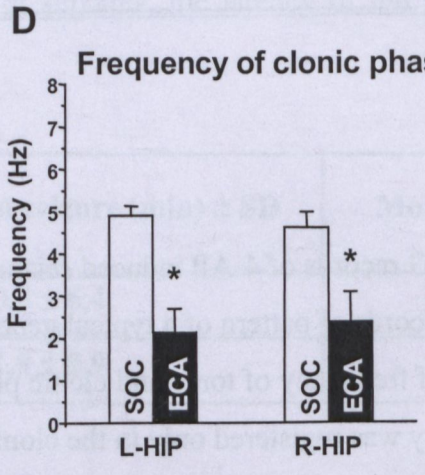
C and **D:** Changes of frequency of tonic and clonic phase in all investigated part of brain. Significant decrease of frequency was registered only in the clonic phase, which was more pronounced in the left hippocampus.

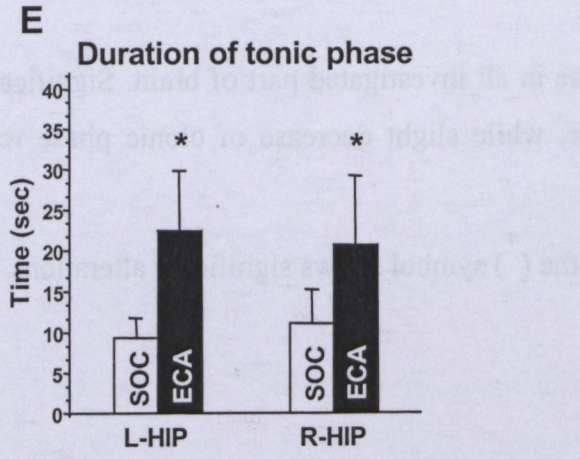
E and **F:** Changes of duration of tonic and clonic phase in all investigated part of brain. Significant increase of the tonic phase was observed everywhere, while slight decrease of clonic phase was recorded.

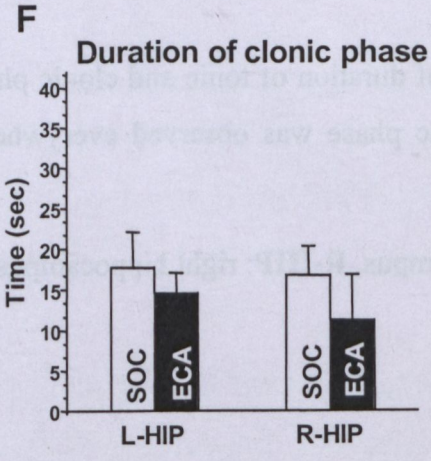
L-HIP: left hippocampus, **R-HIP**: right hippocampus, the (*) symbol shows significant alterations (P ≤0.05).


A
typical seizure from a SOC rat
Initiation
Tonic phase onset
L-HIP
R-HIP
Clonic phase onset
L-HIP
R-HIP
L-HIP
R-HIP
500 μV
2 s
B
typical seizure from an LECA rat
Initiation
Tonic phase onset
L-HIP
R-HIP
Clonic phase onset
L-HIP
R-HIP
L-HIP
R-HIP
500 μV
2 s


C
Frequency of tonic phase
Frequency (Hz)
SOC
ECA
L-HIP
R-HIP


D
Frequency of clonic phase
Frequency (Hz)
*
*
SOC
ECA
L-HIP
R-HIP


E
Duration of tonic phase
Time (sec)
*
*
SOC
ECA
L-HIP
R-HIP


F
Duration of clonic phase
Time (sec)
SOC
ECA
L-HIP
R-HIP

3.4. Changes of neuronal c-fos-expression in LECA during 4-AP seizures

Convulsions induced by 4-AP 40 days after the surgery caused the expression of c-fos in every part of the hippocampus, subiculum, perirhinal and temporal isocortical areas. The expression pattern at 3 h post-injection time was similar to our previous findings on intact animals (39). However, 3 h after the seizure cell counts revealed that the convulsions in EC lesioned animals induced significantly less c-fos expression compared to the control sham-operated rats. The decrease of the number of c-fos positive cells was detected in every hippocampal area (Fig 8 A,B,D). Counting the nerve cell number on hematoxylin-eosin stained sections did not show any neuronal cell death (Fig. 8C).

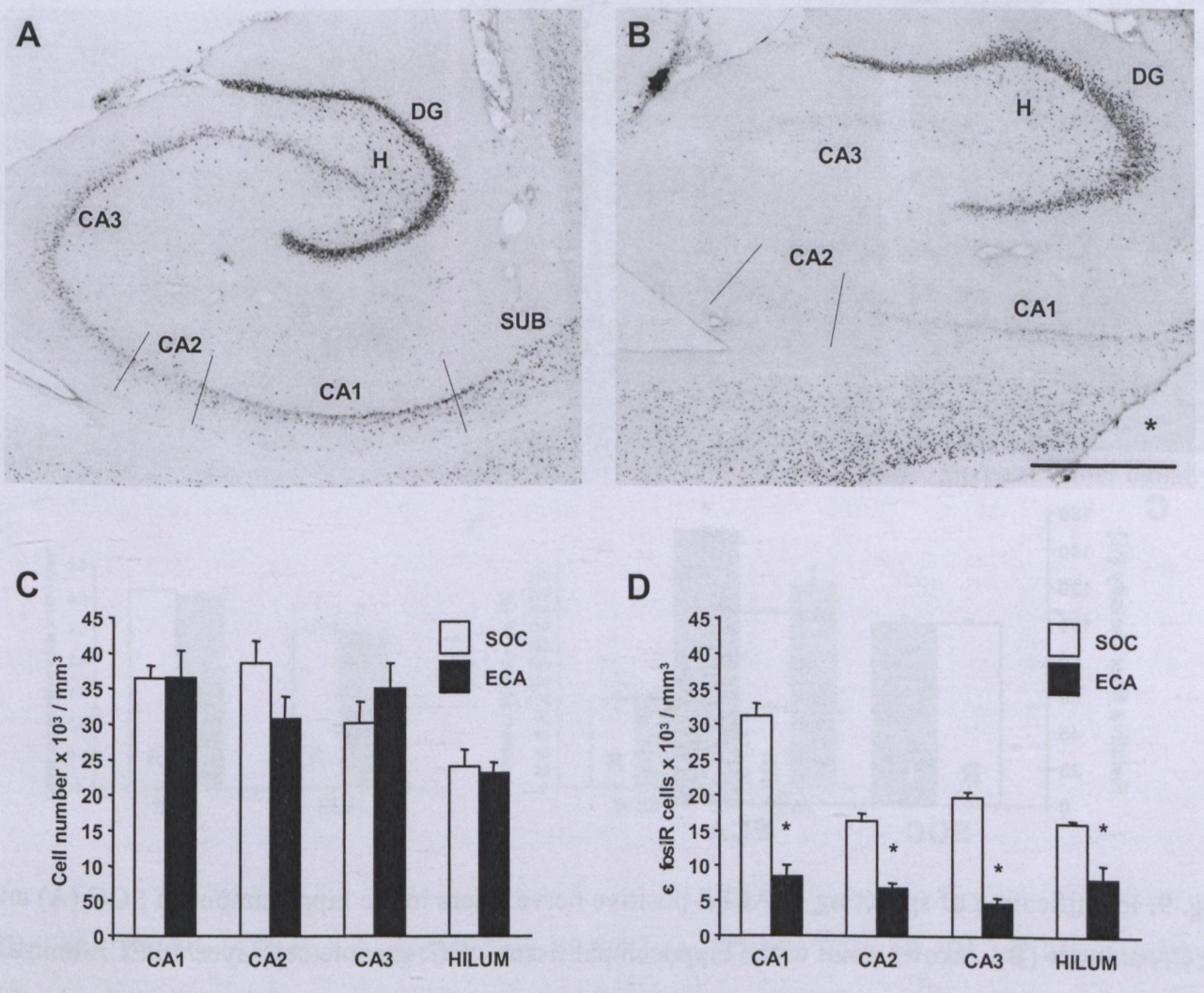


Fig. 8: The animals of EC ablation showed significantly less c-fos expression 3 hours after convulsions in every region examined compared to the control rats **A,B**). H: hilum of the dentate gyrus. Asterisk: EC lesion. Bar: 500μm. **C**: neuronal cell counts did not display differences. **D**: c-fos cell counts display significant differences ($P \leq 0.001$).

3.5. Late histological consequences of EC ablation

3.5.1. Sprouting of cholinergic axons

AChE-positive nerve fibers were detected in every part of the hippocampus. On the side of the ECA, the density of the AChE-positive structures increased significantly only in the outer molecular layer of the DG, where a dense band of cholinergic axons occupied the place of the degenerated perforant path terminals (Fig.9 A and B). Another dense band of AChE staining was seen in the inner molecular layer, just above the granule cells (Fig.9 B).

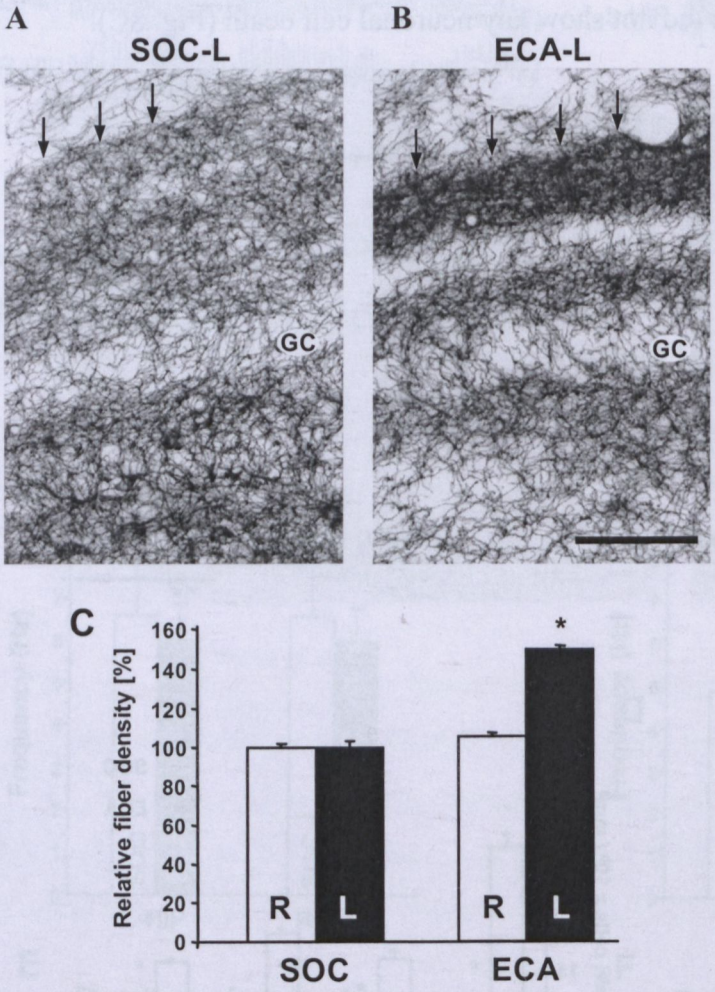
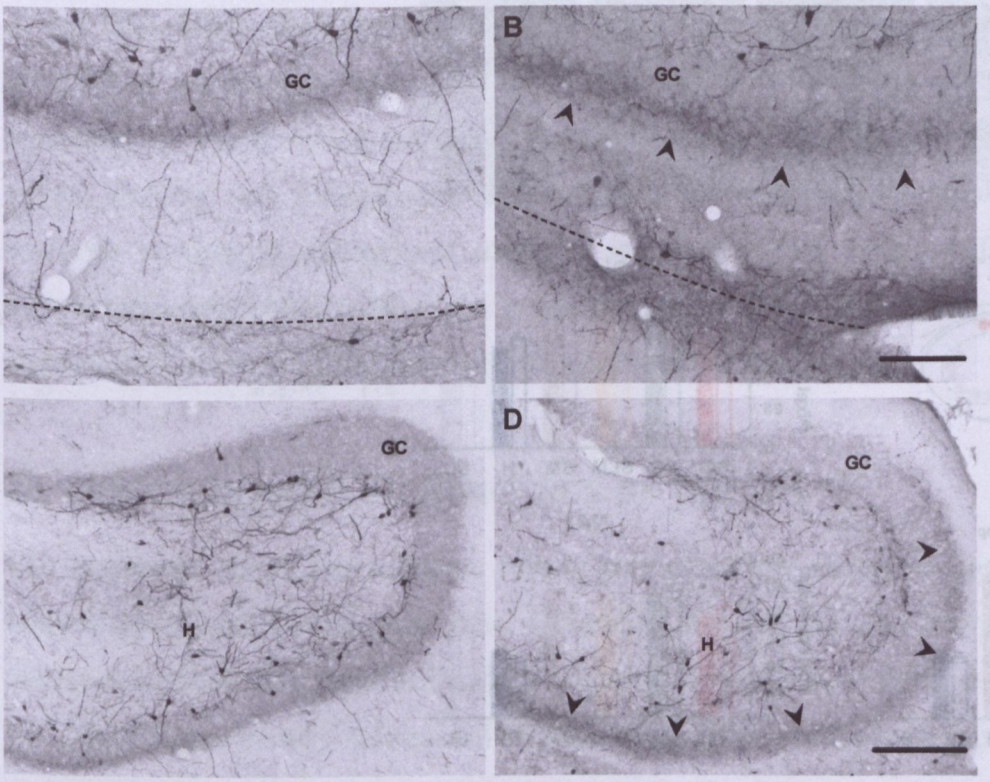
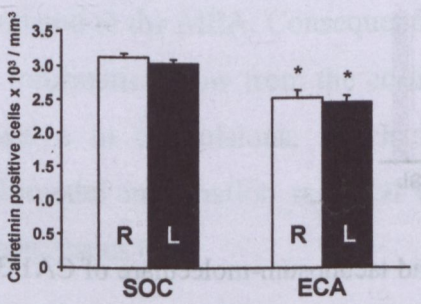


Fig. 9. Identification of sprouting of AChE positive nerve fibers in the hippocampus in SOC (**A**) and LECA animals (**B**). Arrows point to the hippocampal fissure. GC: granule cell layer. In ECA animals (B), the outer molecular layer displays strong AChE staining (arrows). Stronger staining is also apparent at the inner molecular layer (just above the GC). Bar: 100 µm. **C**: densitometry of the molecular layer of the DG indicate a significant (* $P<0.001$) increase in AChE-positive fiber density on the side of the ECA (black columns, L) compared to the corresponding contralateral region (white columns, R). L: left; R: right.

3.5.2. Sprouting of calretinin-containing nerve fibers

Calretinin-positive cell numbers in the DG decreased significantly in the ECA animals, compared to the SOC group. The decrease was significant not only on the operated side, but also in the contralateral hippocampus of the ECA animals (Fig.10 E). Sprouting of the calretinin-positive fibers was obvious, too: in the inner molecular layer of the DG new fibers, penetrating the middle segment of the molecular layer were found (Fig. 10 B and F). However, calretinin-positive sprouting was detected only on the side of the ECA, the contralateral hippocampus did not show increased fiber density.





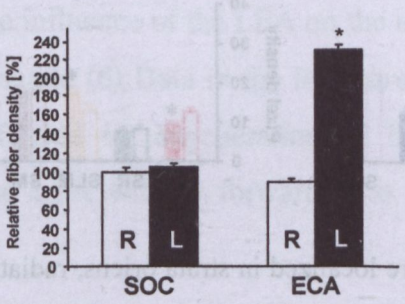


Fig. 10.: Calretinin immunohistochemistry of SOC (**A, C**) and ECA (**B, D**) left hippocampi (H: hilum of the DG; GC: granule cell layer; arrows: hippocampal fissure; bar: 100 μm). Note the shrinkage of the molecular layer (the broken line represents the hippocampal fissure), and the thick, immunostained zone in the inner molecular layer, indicating calretinin positive fiber sprouting (arrowheads). Scale bars: 100 μm for **A, B**; 250 μm for **C, D**.

E: the number of calretinin immunopositive neurons decreased significantly in ECA animals in both hippocampi (*P<0.001). **F**: The relative calretinin fiber density in the whole thickness of the molecular layer increased significantly on the side of ECA (*P<0.001). L: left; R: right.

3.5.3. Changes in the density and distribution of glutamate receptor subunits

Histoblot technics showed the localization patterns of the ionotropic glutamate receptors (Fig. 11).

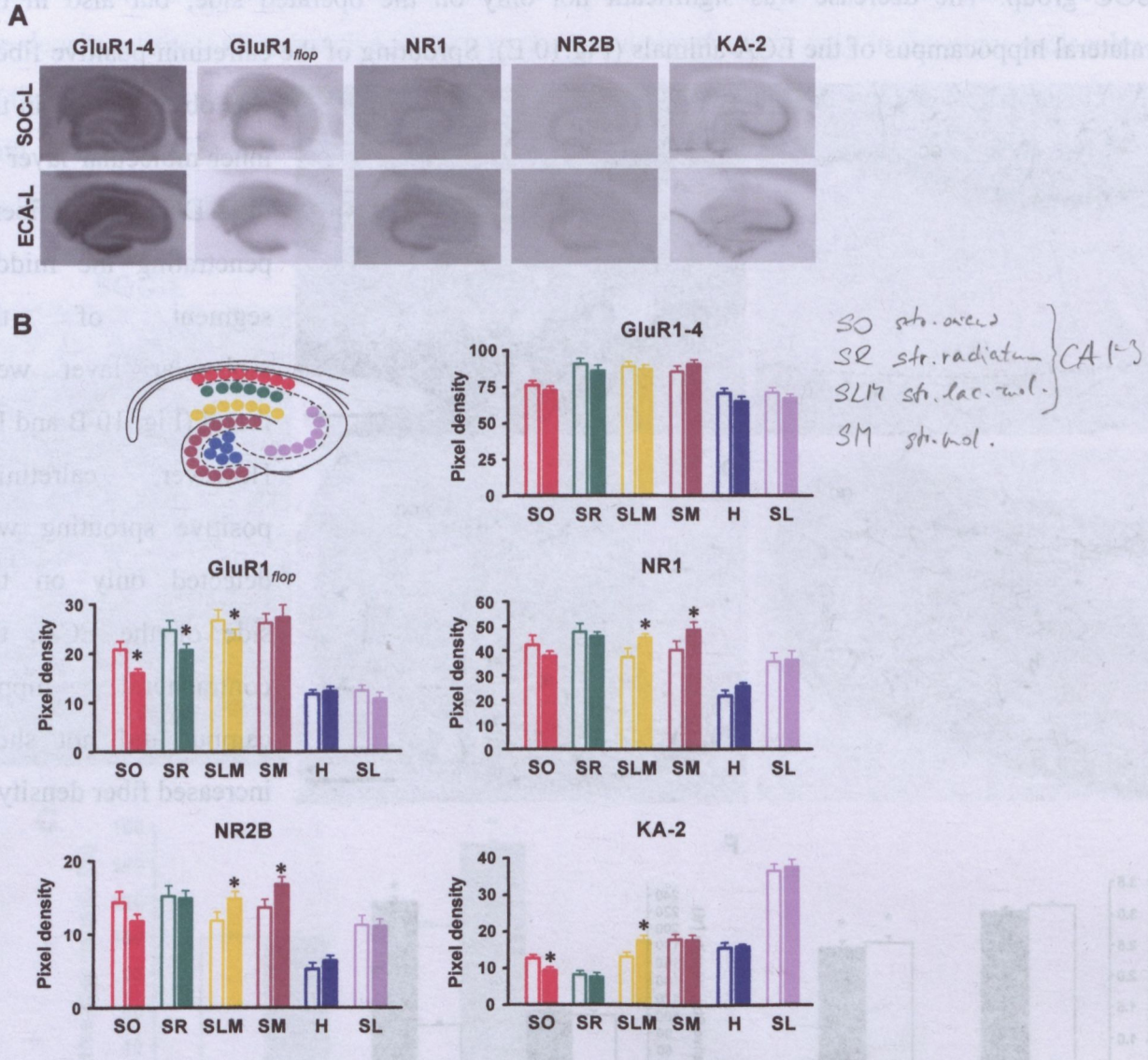


Fig. 11 : AMPA receptors were localized in strata oriens, radiatum and lacunosum-moleculare of CA1-3, and in the molecular layer of the dentate gyrus. Strong NMDA staining was observed in the strata oriens and radiatum of CA1, and in the outer molecular layer of the dentate gyrus. Kainate receptor subunit KA2 was localized mainly in the stratum lucidum of CA3, and in the inner molecular layer (just above the granule cell layer) of the dentate gyrus. No changes of the density of **AMPA (GluR1-4)** staining were detected with histoblotting. Interestingly, **GluR1*flop*** variant displayed significant decreases in every layer of the CA1 region: strata oriens, radiatum and lacunosum moleculare in ECA animals. The staining density of **NMDA** receptors (**NR1** immunostaining) displayed a highly significant increase in the denervated layers: in the molecular layer of the DG, and in stratum lacunosum-moleculare of CA1, on the side of the ECA. The density of immunostaining with **NR2B** antibodies displayed similar increase. The density of **KA2** subunit did not change in CA3, but displayed significant increase in stratum lacunosum-moleculare of CA1, and a significant decrease in stratum oriens of the same region.

4. DISCUSSION

We designed the unilateral ablation of entorhinal cortex of rats so, that we could study different aspects of the surgical intervention. First, we obtained histological evidences of the extent and effect of the LEA lesion, then we recorded the alterations of 4-AP induced seizure. We found that the intensity of neuronal activation decreased, and the mechanism of epileptogenesis compromised, compared to corresponding non-operated rats. Our observations on the spontaneous behaviour and spatial learning also showed clear changes, in terms of increased exploratory activity, decreased reactions to stressful stimuli and impaired spatial mempory capacity. Finally, we demonstrated several phenomena of neuroplasticity in the forms of postlesional sprouting and the rearrangement of postsynaptic ionotropic glutamaterg receptor subunits (I, II).

4.1. THE FUNCTIONAL SIGNIFICANCE OF THE LEA

The entorhinal cortex has a unique role in connecting the neocortex and the allocortex (i.e.: the hippocampus), and in the same time, integrating diencephalic and amygdala-related circuits into these connections. The perirhinal cortex projects preferentially to the LEA: to the layers II and III of EC. These inputs convey multimodal sensory information. Owing to the spatial organization of LEA and MEA, the perirhinal and postrhinal inputs to the LEA are stronger and more concentrated, compared to the MEA. Consequently, the influence of the LEA on the hippocampus is stronger, as to the information flow from the cortical mantle (6). Data in the literature show that the MEA is more sensitive in convulsions, which is reflected by degeneration of layer II/III neurons (9). The multimodal information received by the LEA will be forwarded to the MEA, as well as to the hippocampus (52).

Following our studies the ablation of the LEA effectively destructed the main afferents of hippocampus, without making any direct lesion. So, we have succeded to have an in vivo model of hippocampus deprived of its main afferents, in which hippocampal functions could be studied. The identification of functional, anatomical and molecular consequences of surgical ablation of the LEA was the main focus of our studies.

Considering the central role of EC in the cortico-hippocampal communication, we hypothesized that EC ablation would reduce the seizure susceptibility in the 4-AP induced acut epilepsy model. In fact, our observation of the latency and frequency of the seizures supported this notion. However, we aimed to obtain more insight into the mechanisms of reduced seizure activity. Our studies proved, that the surgical ablation of the LEA effectively destructed the main afferents, without lesioning the

neurons of the hippocampus. So, we have succeded to have an in vivo model in which, the hippocampus has been partially deprived of its main neocortical afferents, and disconnected from an important seizure generator, the LEA (62). We also proved, that this neurosurgical intervention considerably decreased the excitability of the hippocampus. The decrease in seizure susceptibility was proven with EEG, and the immunohistochemical detection of the IEG, c-fos.

4.2. EFFECT OF LECA ON HIPPOCAMPAL FUNCTIONS

As the unilateral ablation of EC significantly reduces intracellular and extracellular electrophysiological phenomena associated to seizure, the benefit of an eventual seizure-controlling neurosurgical intervention can be speculated. Having seen the "beneficial-preventing" effect of reducing epileptogenesis, we wanted to know the "cost" of unilateral EC ablation. We designed our experiments to investigate the effect of unilateral EC ablation on emotional behavior and spatial learning capacities in adult Wistar rats.

The hippocampus and adjacent cortical structures, including the entorhinal, perirhinal, and parahippocampal cortices, appear to serve as an integrated memory system (18, 19, 20, 33, 51, 60). This extended hippocampal system is believed to influence the formation and consolidation of memory traces through an extensive set of reciprocal connections with widespread areas of the neocortex (6, 22, 51). Numerous reports show that lesions to hippocampal afferents, such as entorhinal cortex exert an effect not only on memory but also on other behavioral patterns (e.g.: exploratory activity, locomotion) in rodents (20, 60).

The open field test is based on the conflict between the exploration of a new environment and the aversion to open spaces from which escape is prevented by a surrounding wall. The stimulus of the novel environment may simultaneously induce anxiety and exploratory behavior. We detected an increased exploratory activity as shown by the increased number of horizontal and vertical motion of operated rats. This is in line with behavioral studies performed with rats with bilateral EC ablation or hippocampal lesion. Previous studies using different experimental protocols have shown similar results. Steward (60) detected pronounced but transient increase in open field activity which peaked between 5 and 7 days post lesion and returned to near normal preoperative levels at about 11 days. Our group of rats was about 14-21 days after unilateral EC lesion, however the increase of the exploratory activity was significant. Fass et al. (12) studied the effect of "progressive" (destroying different portions of EC during two occasions of 11-15 days interval) vs. "serial" (performing total EC destruction of different sides on two different occasions) entorhinal lesions on open field activity. They found that "progressive" (but not "serial") lesion enhance the above mentioned

behavioral recovery. They refer to the possible role of spatial and temporal pattern of sprouting in the hippocampus.

The elevated plus maze is widely used as an anxiety paradigm and represents a test based on unconditioned responses to a potentially dangerous environment. The increased time spent in and number of entries in the open arm would be interpreted as a sign of increased exploratory activity but it can refer to decreased anxiety provoked by the stress of the study itself. Steward et al. (60) also performed studies with plus maze to study the consequences of bilateral EC lesions on alternation capacity rather than on emotionality/anxiety-like behaviour. ~~He~~ They observed that EC lesion disrupts alternation performance, probably due to inability to recall which arm was chosen on preceding trial.

The Morris water maze is one of the most common behavioral tasks used to assess spatial (hippocampal) learning and memory in rodents (10, 45). The animal must learn the location of the hidden platform using either distant or local cues. Performance in the Morris water-maze depends on several mechanisms like attention, learning and memory, vision, and motor coordination. Our data did not show difference between the learning process (acquisition) of the two groups, however it did suggest an impairment of spatial memory recall of EC ablated animals. This ~~was~~ has been also shown in animal of selective hippocampal lesions: Galani et al. (16) found that rats with hippocampal damage were impaired in all spatial tasks. However, the rats with lesions of the EC or the subiculum were not impaired in the reference-memory procedure of the water-maze task and showed a deficit in reacting to a nonspatial change. The authors suggest a role for the entorhinal cortex and the subiculum in processing spatial information and indicate a hippocampal-independent role in memory process of the entorhinal cortex.

Short-term memory deficits after unilateral EC lesion have already been presented (18), using Hebb-Williams maze, 7 weeks after electrolytic cortex lesion in Sprague-Dawley rats. In other experiments performed with water maze, EC ablation resulted in deficits of working memory (20). Studies on retention also presented difference of spatial navigation strategy only in the first day. This was interpreted as an incorrect strategy and use of cue integration. We have designed our experiments differently: using adult Wistar rats, the EC lesion was performed 3-4 weeks before to the training period. Our results are in line with those of Glasier et al (19).

Hippocampal place cells have been characterized by location-specific firing that reflects the environment so, that different sensory information can be explored. Place cells use visual and motion-related cues (51). Major part of information coming from the sensory cortex reaches the hippocampus via the EC. The work of Poucet ~~(51)~~ et al. concentrates on the neocortical contribution to place cell spatial firing and demonstrate the relationship between place cell positional activity, and

spatial navigation performance. We hypothesize that the central role of place cell activity in space related memory can offer an explanation to both the increased exploratory activity and the impaired capacity of water maze navigation. Hippocampal place cells present stable, spatially determined, motion-related "firing-fields", which are continuously updated by keeping track of the movements in space based on signals stemming from the vestibular and proprioceptive systems. This mechanism is called "path integration". It is possible that, after EC ablation, the perforant path can not transfer sufficient amount of cortical information toward the hippocampal place cells, which may result in pronounced exploratory activity as a compensatory mechanism. Furthermore, partial deafferentation of hippocampal place cells results in failure of space-related memory functions (18, 19, 20, 51). This can explain the decreased ability in water maze navigation. Altogether, on the basis of our experiments, 3-4 weeks after unilateral entorhinal lesion, only mild deterioration in spatial memory performance occurred. The amygdalo-entorhinal pathway has an important role in processing emotional relevant sensory information, and it is activated during the acquisition of fear-conditioning (32). Manipulation of the neuronal activity of the EC affects emotional learning: lesion of the EC produces anterograde impairment in post-shock freezing (32, 33). The enhanced open field activity and the increased open arm activity in the plus maze test underline the anxiolytic effect of the EC lesion. These results suggest that determining the indication of a potential therapeutic surgical excision of the entorhinal cortex for seizure control needs further pre-clinical and clinical investigations.

4.3. THE CHANGES OF SEIZURE SUSCEPTIBILITY FOLLOWING LECA

Previous studies established that c-fos protein is a reliable indicator of neuronal activity in 4-AP induced seizures (21, 36, 39). The expression of the *c-fos* gene, and the appearance of c-fos protein in the cell nucleus are neuronal activity-dependent processes, and follow a special time course (39, 40, 41). Our recents studies indicate that *c-fos* induction in 4-AP seizures is mediated mainly by ionotropic GluRs (66), and *c-fos* transcription and translation are tightly coupled processes (36). Moreover, c-fos protein immunohistochemistry is widely used in detecting neuronal hyperactivity (39). Our experiments with the rat neocortex proved, that not only the protein, but also the mRNA were synthesized at an elevated rate and level during the 4-AP convulsions. However, we noted, that the c-fos protein appeared earlier, than the mRNA in neocortical neurons. The first brief tonic-clonic convulsion boosted the increase of c-fos protein levels, whilst the second and third convulsions had a strong impact on transcription and translation, as well (36). Neocortical neurons have a low level, basal expression of c-*fos* in normal conditions (41). We think, that the first event in neocortical

epilepsy (during the first seizure) is the translocation of the existing c-fos proteins from the cytoplasm to the cell nucleus. Literature data indicate, that the translocation of c-fos strongly depends on extracellular signals, in our case, enhanced synaptic transmission (56). This translocation stimulates the transcription process, as we have seen the highly significant increase of mRNA levels at 60 min (III).

Following the ablation of the LECA, the overall pattern of c-fos immunoreactivity was similar in both groups, but the quantitative analysis of immunostained cell nuclei in LECA animals revealed significantly less c-fos immunopositive cells in the hippocampus, compared with the SOC group. It is important to emphasize, that the number of neuronal cell bodies did not change in LECA brains, there was no cell loss in the investigated hippocampal regions. The significant reduction in the number of c-fos immunopositive cells was explained with the decrease of neuronal (epileptic) activity. The LECA animals had only one tonic-clonic seizure, which (following the previous arguments of the translocation of c-fos – III) was possibly enough for the stimulation of cytoplasm-to-nucleus translocation of the existing c-fos proteins. Although we did not perform mRNA measurements, we think, that in LECA animals no significant elevation of c-fos transcription occurred in hippocampal neurons.

The other changes, which we monitored with EEG, were the increased latency of the appearance of the first convulsion, as well as the decreased number of overall seizure events, which clearly indicated the increase of the threshold of convulsion in deafferented hippocampi. Changes of the parameters of tonic and clonic phases suggested, that the LEC (through the perforant path) was responsible for the initiation of hippocampal discharges, according to literature data (8).

The capacity of EC to generate epileptiform discharges has been described in *in vitro* electrophiysiological analysis of brain slices, and *in vivo* studies as mentioned in the introduction (62, 63). The inhibitory cells of hippocampus seem to play the main role in eliciting and harmonising the reverberatory activation of neuronal circuits (75). These inhibitory cells may well be activated from the perforant path projections. Consequently, the lengthening of the tonic phase (and the whole seizure) can be explained by the proposed weakening of feed-forward inhibiton. The loss of this GABA-ergic inhibitory projection is due to the absence of perforanth path input, and partially to the degeneration of alvear path (II). As a result, the synchronisation of activated neuronal populations needed to turn from tonic into the clonic phase, is delayed. The decreased number of seizure events is probably due to the loss of the EC, which results in the interruption of spread of neocortical epileptic discharges towards the limbic structures.

All previous *in vitro* studies proved, that the removal of the EC did not abolish the hippocampal seizures completely. Our results are in line with these *in vitro* studies (62). The persistence of the limbic seizures can be explained by two mechanisms. First, the hippocampus has its own CA3-dentate gyrus seizure generator: the CA3 neurons (and mossy fibers) providing excitatory feed-back to the dentate gyrus (31). Thus, the sporadic ictal discharges of the dentate granule cells can reverberate, be enhanced by the CA3-dentate circuit, and turn into a self-maintaining seizure. Second possibility is, that the medial entorhinal cortical area (MEC) generates discharges, which spread to the dentate gyrus through the remains of the perforant path, or through the hippocampal commissure. A seizure originating from the MEC spreads to the dentate granule cells, then to the CA3, from there to the CA1, which projects back to the MEC through the subiculum. The MEC, via the medial perforant path, can re-initiate and maintain hippocampal seizure activity. This seizure is obviously weaker, because the neocortex has been disconnected (6).

Summarising, the ablation of LEC disconnected the hippocampus from the perirhinal cortices, therefore the impact of the neocortical convulsion on the EC-hippocampal circuits weakened. At the same time, LEC ablation disrupted the interaction between LEC and MEC, therefore intraentorhinal reverberation also failed.

4.4. THE REORGANIZATION OF HIPPOCAMPAL SYNAPSES FOLLOWING LECA

Lesioning of the EC protects hippocampal CA1 and CA3 neurons from ischemia- or stress-induced damage, and the interruption of the perforant path can reduce the overexcitation of the DG granule cells by glutamate (28, 64). Both NMDA and AMPA receptors play important roles in mediating over-excitation, since specific antagonists of both types of receptors effectively prevent the epileptiform activity (3).

In this study we have identified region and subtype/subunit specific changes in the expression of iGluR proteins. While the total amount of AMPA receptor subunits remained unchanged, GluR1$_{flop}$ (70) displayed a significant decrease in the CA1 region. Previous autoradiography studies (72) are consistent with our results obtained with the GluR1-4 antibody, which reacts with all AMPA receptor subunit proteins (50). Previous studies reported a small transient decrease in [^{3}H]AMPA binding activity in the molecular layer, three days following unilateral lesions of the EC which returned to control levels between 7-30 days postlesion followed by a moderate increase at day 60 (71). An immunocytochemical study of GluR1 and GluR2/3 AMPA receptor subunits identified no change between days 1-14 following lesioning of the perforant path (42). In contrast, 30-90 days post-lesion, GluR1 immunolabelling was increased in the outer molecular layer of the dentate gyrus

(*i.e.:* in the deafferented zone) ipsilateral to lesion. The GluR2/3 immunolabelling also increased moderately within the same region, although the change was considerably smaller than that which was observed for GluR1 (42). The reported increase in total GluR1 immunostaining (42) may be due to the selective upregulation of the developmentally earlier GluR1$_{flip}$ splice variant in the deafferented regions of the hippocampus. The decrease of GluR1$_{flop}$ immunoreactivity in our experiments may reflect reduced level of activity-dependent recruitment of GluR1 subunits to the deafferented postsynaptic sites. It is generally accepted that in hippocampal neurons the recruitment of GluR1-containing AMPA receptors to the synapse is an activity dependent process, while GluR2 subunits appear to be inserted in a constitutive and activity-independent manner (43, 44). This may explain the more robust changes in GluR1 compared to other AMPA receptor subunits in the deafferented layers of the hippocampus.

At 30-60 days after unilateral entorhinal lesion an increase in NMDA-displaceable [^{3}H]glutamate binding in the dentate gyrus was reported (46, 72). Our study indicates that the increased NMDA receptor binding activity is due to the increased expression of both NR1 and NR2B subunit proteins. Previous quantitative *in situ* hybridization mRNA expression analysis and immunohistochemical comparisons also identified an increase in NR1 at 5-9 days following unilateral transection of the excitatory perforant path (17, 25), which is consistent with our findings. The increased presence of NR2B subunit with relatively slow deactivation kinetics may be a compensatory response of the deafferented neurons in the hippocampus to enhance NMDA receptor-mediated synaptic responses.

Previous studies reported an elevation of the [^{3}H]kainate binding 21-30 days postlesion (46, 71, 72) in areas where we identified increased KA-2 immunoreactivity. These qualitatively similar changes in the topography of [^{3}H]kainate binding, and KA-2 subunit immunoreactivity in the molecular layer of the dentate gyrus are indicative of sprouting of intrinsic hilar associational and commissural input to the dentate gyrus.

Following ECA and perforant pathway lesion some shrinkage of the molecular layer of the dentate gyrus generally develops within the first two weeks (7). Therefore it should be considered that our observations of an increase in NMDA and kainate receptor immunoreactivity may simply reflect an increase in the density of receptor proteins due to reduction in tissue volume. However, the fact that in adjacent sections the GluR1$_{flop}$ and GluR1-4 AMPA receptor subunit immunolabelings demonstrated a decrease and no change respectively, argues against the notion that the observed changes are simply due to shrinkage of the molecular layer of the dentate gyrus. Therefore it is reasonable to conclude that the subunit-specific changes in iGluRs reflect post-lesion reorganisation

of inputs within the deafferented layers of the hippocampus and may be relevant to functional recovery of the damaged circuits.

In conclusion, ionotropic glutamate receptor changes reflected the missing glutamatergic input normally arriving from the EC, mainly via the perforant path. These changes could be due to up- and down regulation or to compensation of reduced function. The exact nature of these phenomena is not yet perfectly understood, however their impact on normal and "epileptic" neuronal function is evident. Once the "meaning" of alterations of receptor subunits will be understood, we will be able to plan antiepileptic medical and surgical therapies much more effectively.

5. CONCLUSIONS AND NEUROSURGICAL OUTLOOK

1. The surgical ablation of the left entorhinal cortex of adult Wistar rats has been proven to be a safe neurosurgical intervention. Animals tolerated the unilateral procedure well, they did not become spontaneously epileptic.

2. The postoperative histological analyses proved, that the surgical excision deprived the entorhinal cortex of its main pathyways, but left the hippocampal structures intact.

3. EC ablation did not cease the 4-AP induced epileptogenesis, however, the c-fos mapping of neuronal networks cleary demonstrated a significant decrease of neuronal activity during the seizure event.

4. EEG studies with chronic brain electrodes provided additional evidences of attenuation of epileptic electric discharges.

5. The LEC ablation increased the exploratory activity significantly, and decreased the sings of anxiety in rats.

6. Partial defects of the spatial memory recall function were also observed, which can be explained by lack of cortical information - normally transferred via the EC - needed for adequate orienation in space.

7. Postlesional neuronal sprouting phenomena, as well as alterations of the expression of different ionotropic glutamatergic receptors reflect postlesional rewiring of afferents and rearrangement of postsynaptic receptor patterns in the hippocampus and dentate gyrus.

The experimental model of unilateral LEC ablation seems to be suitable for the further clarification of the role of the medial and lateral entorhinal areas in the initiation and evolution of epileptogenesis. Also, further thorough electrophysiological and morphological research could shed a light on the

intrahippocampal processes in rats with EC ablation. The the key role of the EC in the epileptic disorders is well accepted (75), during our studies we have succeeded to provide further insight into the special importance of the lateral EC. Nevertheless, major questions are waiting to be answered. A great part of them concerns the relationship between the medial and lateral region of EC, in terms of epileptogenesis. Further modifications of the surgical method, and different epilepsy-models could be tested fo this reason. The other field of further research could be the further "mapping" of synaptic receptors under different conditions. Thourough analysis of the results can help us to understand the epilepsy as a disease of the neuronal membranes, and also to design a comprehensive antiepileptic medical therapy.

Clinical translation of advances in neuroscience regarding epilepsy is an amazing challenge, and should involve radiologists, neurologists, neurosurgeons, and scientists of basic neuroscience. Considerable progress has been achieved in the field of neuroimaging and functional mapping of neuronal structures involved in epileptogenesis (13, 26, 54). Substantial contribution of information technology allowed the implementation of pre-ictal seizure detection and feed-back system, which occasionally enable the patient to prevent the seizures by self-administering antiepileptic drugs. Detailed identification of excitatory and inhibory receptors makes it possible to develop newer and more specific antiepileptic drugs. The molecular-genetic analysis of different types of epilepsies may provide the basic insight into molecular disorders of neuronal membranes.

The neurosurgical implication of epilepsy can also be manyfold. One promising field of therapeutical efforts is the implantation of a stimulator in selected neuronal structures. Stimulation of certain structures can interrupt the evolution or the spread of epileptic discharges. While the stimulation of the vagus nerve proved to be less effective, the thalamic and subthalamic nuclei seem to be more trustful in this regard. Most frequently, however, eplipesy surgery means excision of regions involved in generation or spread of epileptic discharges. The main surgical target is selected by a series of thourough examinations in order to define the epileptogenic focus. Details of the algorythm of the neurological assessment are out of the scope of present thesis, we refer to the extended literature (14, 26).

Our results on a surgically modified experimental epilepsy model, regarding the consequent reduction of limbic epileptogenesis, are encouraging. This evidently raises the question, if a similar neurosurgical intervention could be worked out for patients suffering from drug-resistant epilepsy. In fact, we have to investigate this possibility more in depth, mainly because the EC has a definite role in the normal function of behaviour, learning and memory functions in humans, much stronger than in rodents. Epileptologist and neurosurgeons have to understand more about the mechanisms of

neuronal ciruits involved in seizure so, that the morphological substrate for eventual excision should be strictly delineated, and any damage to normal brain capacities should be avoided.

ÖSSZEFOGLALÁS

Patkányok hasüregébe adott 4-aminopyridin (4-AP) néhány percen belül görcsrohamokat vált ki. Ez a kísérletes idegtudományban elterjedt „akut epilepszia modell" nyújtotta munkánk kiindulópontját. A 4-AP, egy K-csatorna blokkoló, számos hippocampalis excitátoros és inibitoros pályrendszer transzmittereit felszabadítja. A görcs során aktiválódott idegsejthálózat kimutatására a transzkripciót indukáló fehérjék csoportjába tartozó intranukleáris c-fos protein bizonyult alkalmasnak. Az aktivációs hullám terjedésének morfológiai és elektrofiziológiai vizsgálatai során bizonyítást nyert, hogy az entorhinális kéreg képezi a neocorticális régiók és a hippokampusz közötti fő kapcsolatot. Élettani viszonyok között pedig a „limbikus rendszer" részeként az ösztönökkel ill. érzelmekkel kapcsolatos viselkedés kialakításában, valamint a térbeli tájékozódás és memória folyamataiban vesz részt.

Nem találtunk adatot egyoldali lateralis entorhinális kéregirtott (LECA) patkányok elektrofiziológiai vizsgálatáról, a 4-AP indukálta akut rohamok pathomechanizmusáról, valamint az ionotrop glutamáterg receptorok kifejeződésének megváltozásáról. Szintén fel kívántuk térképezni az egyoldali kéregirtás spontán viselkedésre és térbeli tanulásra kifejtett hatásait.

Kísérleteink során felnőtt him Wistar patkányokon a baloldali lateralis entorhinális corticalis régió mikrosebészeti ablációját követően a következő vizsgálati módszereket alkalmaztuk:

1. A kéregirtás közvetlen szövettani következményeit haematoxylin-eosin festéssel, valamint a synapsin-I és a microglia markerek immunhisztokémiai kimutatásával vizsgáltuk.

2. A 4-AP által kiváltott neuronaktivációt c-*fos* immunhisztokémiai módszerrel, az electrophysiológiai jelenségeket mélyelektródás módszerrel vizsgáltuk.

3. A kéregirtást követő idegrostburjánzást és az ionotrop glutamáterg receptorok expresszióját szintén immunhisztokémiai módszerekkel vizsgáltuk.

4. A viselkedés és térbeli tanulás változásait un. „open field", „emelt plus-maze" és Morris féle „water maze"(vízi labirintus) tesztekkel vizsgáltuk.

Vizsgálataink alapján megállapíthattuk:

1. Az egyoldali entorhinális kéreg kiirtását a felnőtt Wistar patkányok jól tolerálják. Spontán kialakuló epilepszia jelentkezését nem észleltük sem klinikai, sem electrophysiológiai megfigyeléseink során.
2. A posztoperatív szövettani vizsgálatok igazolták a bal oldali entorhinális kéreg lateralis areájának műtéti ablációját ill. ennek következtében az. un. perforáns pálya terminális degenerációját az azonos oldali hippocampus területén.
3. Az egyoldali EC abláció ugyan önmagában nem akadályozta meg a 4-aminopyridin provokációra jelentkező generalizált görcsöket, a "c-fos térkép" tanúsága szerint azonban a görcsök intenzitása egyértelműen csökkent az operált oldali temporolimbikus rendszerben.
4. A mélyelektródás EEG vizsgálatok a görcstevékenység gyengülésének részleteiről nyújtottak további információkat. Kimutattuk, hogy az epilepsziás görcsök kialakulásához szükséges mechanizmusok meglassultak ill. megváltoztak. A tónusos fázis tartamának meghosszabbodása és a klónusos fázis frekvenciájának csökkenése egyaránt az epileptogenezis gyengülését mutatta.
5. Kísérleti állataink spontán viselkedése jelentős változásokat mutatott. Az explorációs aktivitás fokozódott és a szorongásos viselkedési paraméterek csökkentek.
6. A Morris vízimedence labirintusban végzett vizsgálatok a térbeli tájékozódás funkciózavarát igazolták az entorhinalis kéreg sebészi ablációját követően.
7. Az AChE és calretinin-pozitivitást mutató idegrostok burjánzása kimutatható a posztoperatív késői időszakban a perforáns pálya terminális degenerációt mutató régióiban. Mindez együtt jár a különböző ionotrop glutamáterg receptorok expressziójának megváltozásával, melyek a postlézionális morfológiai és funkcionális újjászerveződés jelei a hippocampus szintjén.

6. ABSTRACT

Intraperitoneal administration of 4-aminopyridine (4-AP) induces generalised epileptic seizures, originating from the temporolimbic structures. Admitting the central role of entorhinal cortex in limbic epileptogenesis, we performed unilateral entorhinal cortex ablation and examined its morphological, physiological and pathophysiological consequencies.

Our histological studies demonstrate the degeneration of the perforant path, the main pathway connecting the entorhinal cortex to the hippocampus. Decreased seizure-susceptibility of the hippocampus was revealed by means of EEG and immunohistochemistry.

Consequences of unilateral entorhinal ablation on behaviour and spatial memory were studied by means of open field, elevated plus-maze and Morris water-maze tests. These tests showed the increase of spontaneous exploratory activity, with reduced stress responses (anxiolytic effect), and the impairment of the spatial memory recall.

Additionally, we were interested to know more about phenomena of post-lesional sprouting in the hippocampus, likewise the changes of expression of ionotropic glutamaterg receptors in this regard. In fact, our results indicate that a strong axonal cholinergic sprouting occurs following post-lesional degeneration, resulting in a partial redistribution of the expression of glutamaterg receptors.

Our results are discussed in the thesis and a clinical outlook toward a possible epilepsy surgery is given.

The main conclusion are as follow:

1. The left-sided ablation of entorhinal cortex performed on adult Wistar male rats is a safely feasible neurosurgical intervention. Animals generally tolerated well the unilateral procedure, without becoming spontenously epileptic.
2. The postoperative histological analyses revealed the precise extent of surgical excision limited to the lateral entorhinal cortical area. The main pathway originating from the lesioned area was the perforant path, which is supposed to end in the referred hippocampal regions, where signs of secondary degeneration and appearance of microglia proliferation was detected.
3. EC ablation did not supress the 4-AP induced epileptogenesis, however, c-fos mapping of neuronal network cleary demonstrated a decrease of neuronal activation during seizure event.
4. EEG studies of deep brain electrodes gave additional evidences of attenuation of evolution and appearance of epileptic electric discharges, underlying the central role of EC in generating and spreading of seizure activity.
5. Exploratory activity of rats being operated increased significantly, associated with a decrease of sings of anxiety.
6. Partial defects of spatial memory recall function was also observed, which can be explained by lack of cortical information - normally transferred via the EC - needed for adequate orienation in space.
7. The postlesional neuronal sprouting phenomena as well as the modulation of expression of different ionotropic glutamatergic receptors reflect the postlesional rewiring of afferents and rearrangement of receptor patterns in the hippocampus and dentate gyrus.

RÉSUMÉ

Nos études éxperimentales ont été éffectués selon un model de l'épilepsie aigue, induite par la 4-aminpoyridine administrée par voie intrapéritonéale aux rats. Le « cortex entorhinale » étant connu comme une relais entre l'isocortex et l'hippocampus au cours de l'épiléptogenèse, nous nous sommes interessés aux effets de son ablation.

Nos objectifs ont été les suivants :

1 décrire les effets histologiques résultant de l'ablation d'aire entorhinale;

2. préciser le changement de l'activation des groupes cellulaires impliquées dans l'épilepsie aigue induite par 4-AP (refletés par l'expression intracellulaire de la protéine c-fos)

3. décrire les charactéristiques éléctrophysiologiques des crises induites (enregistrement de l'activite éléctrique neuronale au moyen des electrodes intraparenchymal profondes)

4. préciser les changements de l'expression des récepteurs glutamatergiques ionotropiques de type AMPA, NMDA, revelatuers des phenomènes de neuroplasticité

5. décrire les effets de l'ablation du cortex entorhinal sur le comportement spontané des animaux, ainsi que sur leurs capacité de mémoire dans l'espace.

Méthodes utilisées :

ad 1. examens histologiques simples de hematoxyllin-eosin, immunohistochimie mettant en evidence la synaptophysin et marquer de mircroglia.

ad 2. marquage immunohistochimique de la protein « c-fos » intracéllulaire exprimée par les neurones activés au cours des crises comitials.

ad 3. enregistrement avec des electrodes superficielles et profondes placées dans les régions néocorticales ainsi que hippocampales. Examens ont ete éffectués avant et apres l'injection de 4-AP ;

ad 4. mise en evidence immunohistochimique des récepteurs glutamatergiques ionotropiques;

ad5. test de comportement spontané et mémoire dans l'espace: champ ouvert (« open field »), labirynthe élevé de forme +, et labirynthe de type « Morris water maze »

Conclusions principales des éxamens:

1. Les rats tolerent bien l'ablation unilatérale de la région entorhinale du cortex et ne présentent pas de crises épileptiques spontanées.
2. L'éxerèse du cortex entrohinal a provoqué une dégénération secondaire au long the „perforant path" et laissé apparaitre des céllules de type microglia en premier temps, ainsi que des fibres cholinergiques plus tard.
3. L'apparition de protein "c-fos" après les crise comitials induit par 4-AP, étaint nettement diminuée. Par conséquent, l intensité des crises était également baissé.
4. Les études électrophysiologiques également présentaient l'affaiblissement de l'évolution des crises éxperimantales, justifiant le role integral du cortex entorhinal dans l'évolution des crises.
5. Les effet comportemantaux étaient marqués par une activité exploratrice augmentée, ainsi que par des réponses diminuées données aux stimuli stressant.
6. Les déficits des capacites de mémoire spatial étaients également régistrés.
7. Une reorganisation hippocampal de l'expression des recepteurs glutamatergiques était aussi présentée, qui fait partie de neuroplasticité du system limbic dont le fonction n'est pas encore compris.

7. REFERENCES

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